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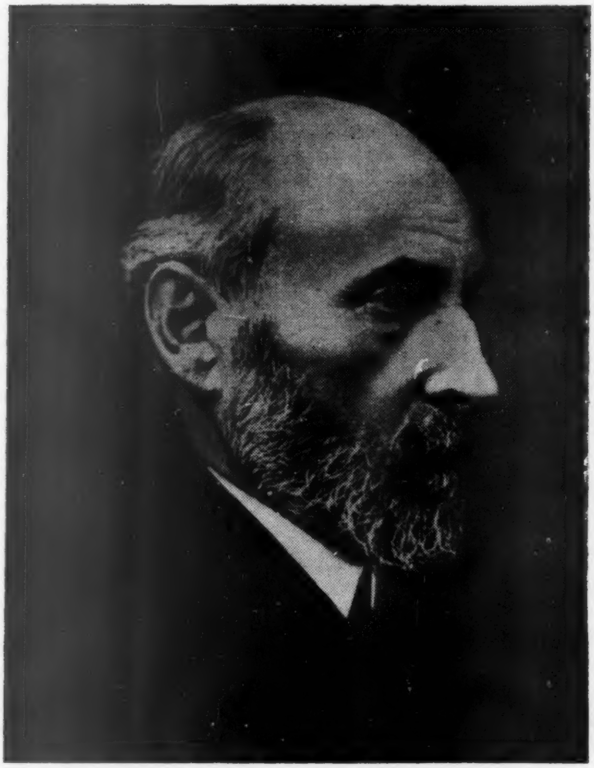
SANTIAGO RAMON Y CAJAL

(1852 - 1952)



La Mesa de Redacción de Acta Physiológica Latinoamericana se adhiere a los homenajes que, con motivo del centenario de su nacimiento, se le han tributado al ilustre sabio español. El comentario que transcribimos a continuación es un resumen de un enjundioso artículo escrito por nuestro colega doctor Clemente Estable, que fuera discípulo de Ramón y Cajal.

HACE cien años nacía en un pueblo de Navarra un niño que había

On occasion of the birth centenary of Santiago Ramón y Cajal, the editorial board of Acta Physiologica Latinoamericana pays its hommage to the great spanish scientist by inserting this note which is a summary of a very important and substantial article written by our colleague Dr. Clemente Estable, who was one of his pupils.

A HUNDRED years ago a boy who would be to spanish science what

de ser a la ciencia española lo que Cervantes a las letras, don Santiago Ramón y Cajal. Después de una juventud desordenada en la que se manifiestan tendencias variadas —la de explorador de la naturaleza, la de conquistador, la de pintor y la de aventurero— Cajal encauza su vida en la ciencia tanteando primero direcciones nuevas en la histología y la bacteriología. Conoce la técnica de Golgi a través de Simarro en el año 1887 dándose cuenta clara de la fecundidad de dicha técnica. Tuvo la genial idea de aplicarla al embrión, lo que le permite realizar su primer descubrimiento trascendente en el año 1888: la sinapsis interneuronal. Los hechos y las ideas del investigador español revolucionaron la anatomía del sistema nervioso y pusieron orden donde existía una confusión. Sus investigaciones demostraban que no existía continuidad sustancial entre célula y célula y que, por consiguiente, el impulso nervioso debería trasmitirse por contacto o por algún modo de conducción. Las investigaciones más importantes y originales desde 1888 a 1903 se concretan en una obra maestra: *"Textura del sistema nervioso del hombre y los vertebrados"*.

Hasta entoces su instrumento de trabajo había sido sobre todo la técnica de Golgi del cromato de plata, perfeccionada y usada según estrategia personal; su obra es de morfología neuronal, de conexiones entre neurona y neurona, tectonica de los centros y de los ganglios nerviosos y de neurogénesis. Es descriptiva con indicaciones funcionales y generalizaciones admirables.

Las publicaciones de Apathy (1897) y de Bethe (1900) provocaron un movimiento de progreso al poner en evidencia las neurofibrillas; pero la técnica por ellos empleada revelaba

Cervantes was to spanish literature was born in a small village of Navarra. Don Santiago Ramón y Cajal had a rather disorderly youth in which various tendencies dominated him temporarily: explorer of nature, conqueror, painter, adventurer. But at last he devoted himself to science trying first new paths in bacteriology and histology. Acquainted with Golgi's technique through Simarro in 1887, he immediately grasps its fertility. The brilliant idea of applying this technique to the embrio leads him in 1888 to his first far-reaching discovery: the inter-neuronal synapsis. The facts discovered by the spanish researcher and his ideas brought a revolutionary change in the anatomy of the nervous system and brought order where confusion reigned. His work showed that there was no substantial continuity between nervous cells and consequently the nervous impulse had to be transmitted by contact or by some other method of conduction. His more important and original investigation from 1888 to 1903 took form in a masterpiece: *"Texture of the nervous system of man and vertebrates"*.

Up to 1903 his working tool had been Golgi's silver chromate technique, modified and used with personal strategy. His work is about morphology of the neurone, interneuronal connection, texture of nervous centers and ganglions and neurogenesis. It is descriptive with functional inductions and admirable generalizations.

The papers of Apathy (1897) and Bethe (1900) signified a progress by showing the existence of neurofibrilles; but the technique they used re-

estructuras que parecían contradecir la doctrina de la neurona, dando lugar a la tesis de que las neurofibrillas atravesarían las células nerviosas para rematar en un retículo intercelular. Preocupó a Cajal el problema y se dió cuenta de que el edificio teórico construído por Bethe estaba basado en una técnica imperfecta. De regreso de un viaje a Italia surge repentinamente en su mente una hipótesis que explicaría los fracasos del método de Simarro (1900). Evoca Cajal su revelación en estos términos: "La substancia enigmática generadora de la reacción neurofibrillar debe ser pura y sencillamente el nitrato de plata caliente incorporado a los coloides del protoplasma y susceptible de precipitarse en estado coloidal y en virtud de procesos físicos sobre el esqueleto neurofibrillar". Esta idea se apoderó de él como una obsesión. Dice Cajal: "Devorábame la impaciencia, y ansiaba hallarme en el laboratorio para poner en práctica mis proyectos... A mi llegada a Madrid, caí sobre los animales como león sobre su presa...".

Y así surgió, a partir de la técnica de Simarro, la mejor técnica neurofibrillar que poseemos. No existe territorio del sistema nervioso donde Cajal no haya cavado hondo. De singular importancia es su aporte al problema de las localizaciones, vías y centros. En cuanto a las del cerebro, he aquí su autojuicio: "Mis preparaciones mostraron una urdimbre específica y absolutamente inconfundible, quedando así sobre bases histológicas inconmovibles la doctrina a la razón muy discutida de las localizaciones cerebrales". La escuela alemana con M. Rose reconoce que la descripción básica de la corteza visual hecha por el sabio español "es, hasta ahora, la mejor que poseemos". Y lo mismo puede decirse de la corteza acústica y de la corteza sub-occipital.

vealed structures which seemed to contradict the doctrine of the neurone and gave way to the thesis that the neurofibrilles went through the nervous cells to end in an intercellular reticule. Cajal was concerned with this problem and he reckoned that the theory built by Bethe was based on an imperfect technique. Returning from a trip to Italy, a hypothesis suddenly rises in his mind which would explain the failures of Simarro's modifications of Bethe's technique. Cajal himself reports his revelation in the following terms: "The enigmatic substance which generates the neurofibrillar reaction must be simply the hot silver nitrate incorporated to protoplasmic colloids and which precipitates in a colloidal state due to physical processes on the neurofibrillar skeleton". This idea dominated him like an obsession. He says: "I was consumed by my impatience... and was anxious to be back in my laboratory so as to put in practice my plans... On arriving to Madrid I fell over my animals as a lion over his prey..." And thus came forth the best technique available for neurofibrilles. No territory of the nervous system was left by Cajal's deep exploration. His contribution to the problem of localizations, centers and nervous pathways was of particular importance. In his own words "My preparations showed a specific and absolutely typical configuration which gave unyielding histological basis to the much discussed doctrine of cerebral localizations". And the german school recognizes with M. Rose that the basic description of the visual cortex made by Cajal "is until now the best we have". The same could be said

En el inmenso horizonte de la neurología está la mayor presencia de Cajal. Su obra incomparable se levanta sobre firme base descubierta en hondas exploraciones sobre el sistema nervioso. Se desarrolla en tres cuerpos como un monumento imponente: en el centro las investigaciones sobre la tectónica del neuroeje y de los ganglios, la morfología y la sinapsis neuronales; en una de sus alas los estudios sobre neurogénesis; en la otra los estudios sobre degeneración y regeneración del sistema nervioso. Como bajo relieve la glía y las neurofibrillas en su estática y en su dinámica. De la genial doctrina de la neurona, con iluminación fisiológica, surge la bella y magnífica unidad del monumento.

Cajal era un infatigable trabajador. "Entre uno de mis defectos, confiesa, acaso el más grave fué siempre la falta absoluta de método y mesura en el trabajo." Para él, el que no trabaja no existe. Otra de las características del sabio español es su voluntad. Enamorado de los temas cuya investigación emprendía, dirigía todos los poderes del espíritu al mismo objeto. Fué un gran animador de la investigación científica en España y en Hispano-América. Agudo observador y experimentador sagaz, sigue escrupulosamente todas las sinuosidades e incidencias de la realidad y jamás desdeñará un hecho por una hipótesis. Vigoroso y sutil razonador, a la vez que intuitivo, estima como don sagrado la voluntad y la plasticidad y sin pena cambia de opinión cuando el cambio es de un error por una verdad, sea o no el errror suyo y la verdad de otro.

La vida y la obra de Cajal son un estímulo para el mundo de habla hispana y un ejemplo para el mundo entero.

of the acoustic and sub-occipital cortex.

In the wide field of Neurology, the personality of Cajal is prominent. His monumental work rises in the firm base of sound investigations of the nervous system. His works in the texture of the neuroaxis and the ganglions, the morphology of the neurones and its synapsis, his studies on neurogenesis and on degeneration and regeneration of the nervous system: his descriptions of the glia and the neurofibrilles are his greater contribution to science. And all this work is unified and integrated by his wonderful conception of the neurone.

Cajal was an untiring worker. "I must confess that perhaps the most weighty of my imperfections has been the absolute absence of method and moderation in my working habits". "Those who do not work do not exist", he used to say. Another of his characteristics was his will power. He literally fell in love with the subjects chosen for investigation and all his spiritual powers where projected on them. Acute observer and skilled experimenter he followed very carefully all the sinuosities and incidences of reality and never disdained a fact for a hypothesis. Vigorous and keen reasoner, and at the same time an intuitive, he estimated will power and plasticity as God sent gifts and did not hesitate to change his opinion when change implied accepting a truth instead of an error, without considering if the error belonged or not to him and the truth to someone else.

The life and work of Cajal are a stimulus to the spanish speaking world and an example for the whole world.

INHIBITORY ACTION OF THE MOTOR NERVE ON THE STRIATED MUSCLE

RAÚL CARREA M. D., CARLOS C. DEVOTO M. D., ALFREDO LANARI M. D.

(Cátedra de Patología y Clínica de la Tuberculosis. Facultad de Ciencias Médicas. Buenos Aires)

IT IS well known that the neuromuscular synapsis of the striated muscle becomes hypersensitive to acetylcholine after motor nerve section (Frank, Nothmann and Hirsch-Kaufmann, 1923). In 1936, Lanari observed that in hemiplegic patients the muscles of the paralyzed limbs become sensitized to intraarterial acetylcholine. It has also been demonstrated that partial denervation of the spinal motoneurones, by spinal hemisection, causes a hypersensitivity of the spinal motoneurones and of the neuromuscular synapsis to the acetylcholine injection (Cannon and Haimovici, 1939). The development of hypersensitivity of the spinal motoneurones and of the neuromuscular synapsis after spinal hemisection is only noticeable two days later and is completely evident within 5 to 14 days.

In the course of investigations made for the purpose of establishing which long spinal fibres could be responsible for the sensitization of the spinal motoneurones and of the neuromuscular synapsis after partial denervation, we found that the results of intraarterial or intravenous injection of acetylcholine were more conspicuous when the motor nerve was sectioned. This led us to inquire if this same phenomenon would take place in the intact animal, since these first results showed a hypersensitivity to acetylcholine injection *immediately* after nerve section.

As a precedent related to this investigation in crustacea, Katz (1936), Welsh and Schallek (1946) and several others (Katz and Kuffler, 1946), described a double innervation: motor and inhibitory. The motor response is abolished by stimulation of the inhibitory nerve. The inhibitory axon when stimulated alone does not provoke mechanical response but a small intramuscular nerve spike could be detected. In other species

McIntyre (1947) and McIntyre and Bennett (1949) have contributed facts which suggest an inhibitory effect of the intact motor nerve.

Material and methods

The studies were carried out in 60 adult cats and 5 adult dogs. 30 cats were normal; in 12 the pyramids were cut in the medulla oblongata (on both sides in 10 and on one side only in 2) exposed with aseptic technique by an anterior parapharyngeal approach, 5 to 27 days before; in 3 the patellary tendons were sectioned 6 to 20 days before; on another 3, lesions of the cerebellum were made; and spinal hemisections were made on two. In 5 dogs the thorax was opened and the midthoracic aorta was closed during 20 to 22 minutes, the records were obtained 6 to 25 days later. A neurological record was kept of all the animals and in many of them the motor disturbances were recorded cinematographically. Immediately after killing the animals necropsy was made and specimens hardened for the histological study of lesions and of possible secondary degenerations.

The majority of the experiments in the cats were carried out with intraperitoneal anesthesia with Dial "Ciba" (0.80 ml per kg of body weight). In a few cases the animals were decerebrated. For the dogs, on the other hand, intraperitoneal chloral morphine was used. A tracheal cannula was placed in all animals, but in none of them artificial respiration was necessary.

The records were made using the quadriceps or gastrocnemius muscles. The muscular tendons were attached to the myographs by means of rigid strings and the muscles contracted against the weak resistance of one or two thin rubber bands. The heterometric rather than heterotonic contractions were recorded by means of a lever in a kymograph of variable speed. Nearly always the contractions of two symmetrical muscles were recorded simultaneously, sometimes successively. Except when otherwise indicated the amplification was 5 times. In all the cases the femoral and/or sciatic nerves were exposed (care being taken of not harming the nutrient vessels) and surrounded with a string so as to be able to expose them without any delay when desired.

The injections of acetylcholine (10.1 and 0.1 mg per cm^3) or of 5 % potassium chloride (preceeded by atropinization at the rate of 1 mg per kg of body weight, and the removal of the adrenal glands) were made through a Lindemann needle inserted in the internal jugular vein, in the femoral artery, according to the technique described by Rosenblueth, Lissak and Lanari (1939), in the iliac artery of the opposite side or, more frequently, in the abdominal aorta through one of the renal arteries.

In cases in which electrical stimulation of the motor nerves was used, large bipolar shielded electrodes were placed around the sciatic or femoral nerves, according to the case. A thyratrone stimulator of variable frequency and amplitude was usted. Both nerves were stimulated

at the same time with supramaximal discharges and previous testing of poles.

OBSERVATIONS

1) *Normal Cats.* — In the normal adult cat novocaine nerve block or motor nerve section causes, in the majority of the cases, an increase of muscular response to the intravenous or intraarterial injection of acetylcholine or potassium (fig. 1). The small amplitude of this response requi-

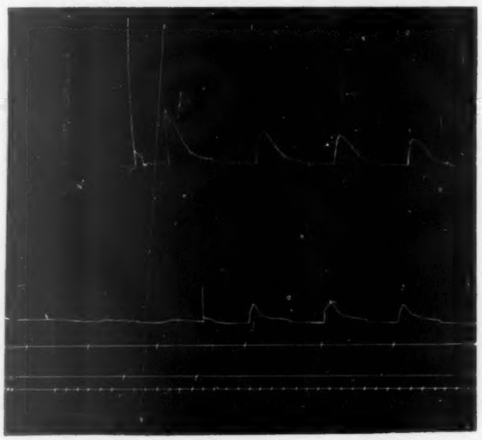


FIG. 1. — *Normal cat. Above: right gastrocnemius muscle; below: left gastrocnemius muscle. Amplification: 22 times. Upper sign: acetylcholine, 7 mg, retrograde injection through renal artery every 4 minutes; lower sign: first, right sciatic nerve section; second, novocainization of left sciatic nerve. Time: every 15 seconds Graph interrupted during 2 ½ minutes between injections.*

res for its demonstration a large amplification of the myograph (i. e. : 22 times). It is greater when the injection is given through the abdominal aorta by way of the renal artery and when the nerve that innervates the antagonistic muscles of those explored has been previously sectioned. It must be added that the potassium response is rapidly exhausted, while the acetylcholine response persists, which could be due to the general toxic effect of the first drug.

Hypersensitivity to acetylcholine or potassium-chloride following sciatic nerve section normal cats.

	Evident	*Doubtful*	*Absent*
Previous section of femoral nerve	10	1	0
Intact femoral nerve	2	4	3
TOTAL	12	5	3

2) *Cats with cerebellar lesions.* — In animals with lesions in the interior of the cerebellum plus a small lesion of the brain stem, which

caused an increased resistance to the passive movements of the limbs with certain characteristics similar to decerebrated rigidity (such as was observed in the primates by Carrea and Mettler, 1947) a hypersensitization is produced to acetylcholine injections similar to that described by Cannon and Haimovici (1939) in spinal hemisections, so that a 5 mg injection of acetylcholine in the jugular vein promotes, with normal amplification, a noticeable response. Fig. 2 shows a myograph of a cat in which a cerebello-medullary lesion had been produced 5 days before. The response, seen in the lower record, is that of a muscle which had been denervated about an hour earlier: the height and persistence of the

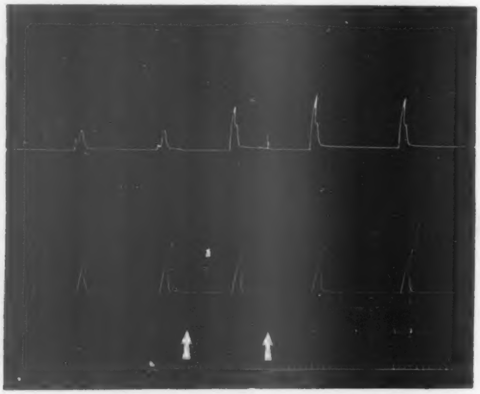


FIG. 2. — *Cat with a cerebellar lesion 5 days before. Above: left quadriceps muscle; below: right quadriceps muscle. Upper sign: 5 mg injection of acetylcholine through the external jugular vein, every 3 minutes. Right crural nerve previously sectioned. First arrow: novocainization of left crural nerve; second arrow: left crural section. Time: every 15 s.*

response is remarkable. In the upper record the response obtained with the intact motor nerve, is seen; the hypersensitivity due to the cerebello-medullary lesion is pointed out. By blocking the femoral nerve on that side with novocaine, without eliciting any muscular jerk as a result of mechanical stimulation, the muscle response definitely increases at the following acetylcholine injections. A slight muscle jerk is produced when the femoral nerve is cut, which shows that some of the nerve fibers had not been blocked by the novocaine, and it is observed that after nerve section the amplitude of the response to the acetylcholine injection is even greater. Results as remarkable as these were found in the 3 animals with lesions of the cerebellum and medulla oblongata.

3) *Tenotomized cats.* — In 3 cats the patellary tendon was sectioned 6, 12 and 20 days before obtaining the records. In the 3 cases the indirect stimulation with different frequencies gave results similar to those observed by Thomsen, Luco and Altamirano (1942). With intravenous acetylcholine injection, the hypersensitivity of the neuromuscular synapsis was not very evident in an animal that had been tenotomized 6 days

before, while it was more noticeable in the other 2 animals, that had been tenotomized 12 and 20 days respectively. In these last 2 the motor nerve section greatly increased the response to the acetylcholine injection. As can be observed in fig 3, this phenomenon is only an exaggeration of what is observed in the normal muscle (compare upper graph to lower graph).

In one animal it was observed that indirect tetanic stimulation of the muscle caused an increase of sensitivity to the acetylcholine injection. This hypersensitivity was, however, transitory and was lessened after three or four injections of the drug. On the other hand after motor nerve section, the acetylcholine response was of greater amplitude, was permanent, and could in its turn be potentiated with tetanus elicited through indirect stimulation of the muscle.

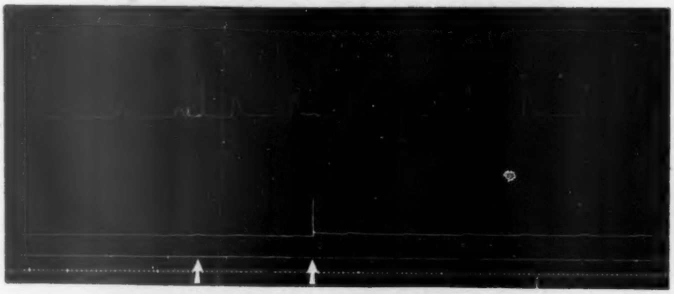


FIG. 3. — *Cat with left patellary tendon section 20 days before. Above: left quadriceps; below: right quadriceps. Upper sign: 10 mg injection of acetylcholine through external jugular vein every 3 minutes (the 7th injection of the graph was of 5 mg of acetylcholine, approximately). First arrow: left crural section; 2nd. arrow: right crural section. Time: every 15 s.*

4) *Dogs with occlusion of the thoracic aorta.* — In 5 dogs the thoracic aorta was clamped during 20 to 22 minutes. As a result of such procedure the spinal blood supply below the level of the occlusion is compromised, and a paraparesis appears, which draws our attention because of the spasticity of the limbs. The intensity of the paraparesis is, however, variable. In 2 of these animals the paraparesis was very slight, in another 2 it was very evident, and in the last one it was very noticeable and with very marked spasticity. The gastrocnemius muscle was used for the myograms and the injections of acetylcholine were given in the external jugular vein in these 5 cases (fig. 4).

The results of indirect stimulation with increasing frequencies, and of intravenous acetylcholine injection, in these spastic animal with an otherwise intact nervous system, and anesthetized with chloral-morphine, vary according to the degree of paraparesis, and to the time that has elapsed after the occlusion of the aorta. The responses obtained are generally asymmetrical. Hypersensitivity to intravenous acetylcholine injection can be seen. When this hypersensitivity is asymmetrical, an advance

in the synapsis of the side more sensitive to the acetylcholine is observed, while the myotatic reflexes are more active on the opposite side. The increase of the response to the acetylcholine injection immediately after motor nerve section is proportional to the degree of previous hypersensitivity to acetylcholine, and it could be observed in 4 out of 5 animals.

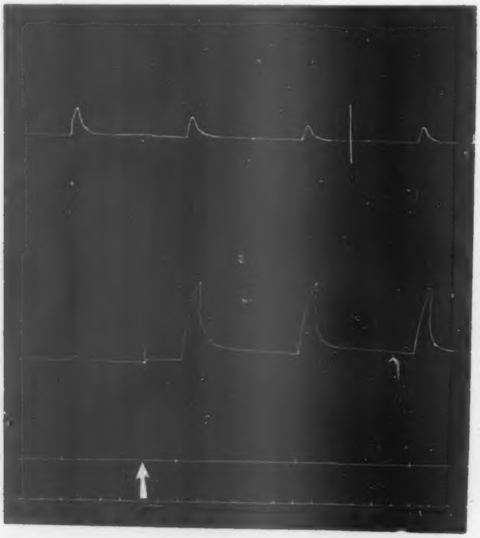


FIG. 4. — *Dog with paraparesis caused by occlusion of toracic aorta during 20 minutes 10 days before. Above: right gastrocnemius muscle; below: left gastrocnemius muscle. Upper sign: 5 mg injection of acetylcholine through external jugular vein. Arrow: left sciatic section. Time: every 30 s.*

5) *Cats with section of the pyramids in the medulla oblongata.* — If one or both pyramids of the medulla oblongata are sectioned, a preparation is obtained in which the pyramidal impulses transmitted to the spinal cord are absent. The lesion is strictly confined to the pyramids since the section can be effected without damaging blood vessels or adjacent nervous structures. Slight differences in the physiological results of this lesion on one side or the other, can be attributed to different levels in which the lesion was placed. In ten out of 12 animals records were made with the brain intact and dial intraperitoneal anesthesia, while in the other two animals decerebration was carried out before obtaining the records.

In the cat, bilateral section of the pyramids causes motor disturbances similar to those described by Tower (1940) in the primates but less remarkable: at no time do the animals remain paralyzed. A discrete quadriparesis with moderate and inconstant spasticity is produced and, as well as an exaggeration of the myotatic reflexes, there is a remarkable tardiness in the initiation and a sluggishness of movement: the animals

sometimes walk as if they were being seen through a slow motion picture, rarely do they chase or run away, a motor hypoactivity being evident. Such as in the case of "partial denervation of the spinal motoneurons by spinal hemisection", in these cases a hypersensitivity is observed of the neuromuscular synapsis to acetylcholine and potassium intravenous or intraarterial injection, and possibly also of the spinal synapsis in relation to the pyramidal motor system. Here it should be remembered that, as Lloyd (1941) has shown, the axons of the pyramidal system do not directly

FIG. 5. — *Cat with section of medulla oblongata pyramids, 7 days before. The left one in the bulboprotuberantial limit, the right one 4 mm below. Above: left quadriceps; below: right quadriceps. Upper sign: 3 mg injection (10 mg in the last 3 injections) of acetylcholine through external jugular vein, every 3 minutes. 1st. arrow: left crural section; 2nd. arrow: right crural section; 3rd. arrow: novocainization of the distal portion of the right sectioned crural. Time: every 15 s. Note that after left crural section the response of the left quadriceps, as well as increasing the amplitude, precedes that of the right quadriceps by 15 s; both responses become synchronical again when both nerves are sectioned.*

activate the motor neuron of the anterior motor horn but the internuncial neurons whose bodies are situated in the dorsal horns of the spinal cord.

A week after operation the pyramidotomized cat (anesthetized with Dial) is an excellent specimen to study acetylcholine hypersensitivity immediately after motor nerve section. The analysis of figs. 5 to 8 inclusive, and of the synopsis, clearly illustrates the intensity and persistence of this phenomenon.

FIG. 6. — *Cat with section of medulla oblongata pyramids 3 mm below the bulboprotuberantial limit, 8 days before. Record of left gastrocnemius muscle. Amplification: 13 times. Upper sign: 3 mg retrograde acetylcholine injection through the renal artery, every 4 minutes. 1st. arrow perineural novocainization of the left sciatic 2nd. arrow: left sciatic section. Time: every 30 s. Record interrupted during 2 m (approximately) between injections.*

FIG. 7. — *Cat with section of the medulla oblongata, the left one in the bulboprotuberantial limit, the right 4 mm below, approximately, 9 days before. Above: right gastrocnemius muscle; below: left gastrocnemius muscle. Upper sign, short signs: 4 mg injections of acetylcholine through external jugular vein, every 3 m (except the 12th injection, which was given 2 m before the last one and the 14th injection, which was given 4 m before the previous one, the last 2 injections were of 13 mg of acetylcholine). Prolonged signs: stimulation of both sciatic nerves with current of 200 cycles per s during 15 s. 1st. arrow: left sciatic nerve section; 2nd. arrow: right sciatic nerve section. Time: every 15 s. Note: a) this previous postetanic potentiation of the sciatic nerve occurs when the nerve stimulation is carried out immediately before the injection, and it ceases after a few minutes; b) that the potentiation of the response to the acetylcholine which follows sciatic nerve section, occurs even when the section has been performed for 1 ½ to 2 m before the injection and persists without modification during all the recording (56 m to the left, 40 m to the right); c) after the left sciatic nerve section the response to the left precedes the right while on sectioning the other sciatic nerve, the responses becomes synchronized again.*

Being such that when the motor nerve is sectioned a muscular jerk is produced, it could be objected that the ulterior potentiation of the acetylcholine injection response could arise from an analogous phenomenon as that of the postetanic increase of acetylcholine responses, as has been described by Rosenblueth and Luco (1937), (see fig. 8, page 787), among others. Both types of phenomena can be differentiated in the experiment illustrated on fig. 7.

FIG. 8. — *Cat with section of the medulla oblongata pyramids 8 days before. - Quadriceps muscle. Arrow: 10 mg retrograde injection of acetylcholine, through the iliac artery on the side opposite the explored muscle without stopping the circulation. In 1, with intact crural nerve; in 2 and 3, respectively, 2 m and 8 m after crural nerve section. Time: every one second.*

On the other hand, the phenomenon described is equally observed, as has been seen in other experiments (see above), by blocking the motor nerve with novocaine. In such case the ulterior response to acetylcholine injection is potentiated without having produced a muscular jerk. More so, if the effect of novocaine wears out the potentiation produced by the block disappears, only to reappear if the nerve is then sectioned. This would indicate that the phenomenon is reversible, as can be seen on fig. 6. On the other hand, if the novocaine block is partial, the potentiation caused by the block is not very great, which is evident if it is compared to the potentiation which follows complete block by severance of the nerve or through novocainization (figs. 1, 2 and 6). The degree of potentiation would therefore be proportionate to the percentage of fibers blocked or to the quality of the fibers blocked in the first place. In an experiment which has not been repeated we have been able to observe that reversible potentiation can also be achieved by blocking the nerve producing an anelectrotonus.

That potentiation results not only from an increase of the amplitude of the response but also from a shorter latency can be readily shown increasing the speed of the recording drum, as seen on fig. 8.

Additional factual data can be taken from the analysis of the synopsis of the experiments carried out on pyramidotomized cats. (table I).

DISCUSSION

The experiments described, clearly show that each time that the motor nerve is sectioned or blocked, there is a marked increase in the muscular contraction which follows the intravascular injection of acetylcholine or potassium. This phenomenon is evident in normal animals only if the amplification of the myograph is increased, but can be clearly seen

TABLE I

Synopsis of experiments in pyramidotomized cats

Animal Number (cats)	Side of pyramidal section	Days elapsed after section of pyramids	Anesthesia	Motor nerve of antagonistic muscle cut.	Injection performed			Hypersensitivity to the drug injected			Figures
					Drug	Vía	Dose mg	Previous to motor nerve section	Following novocaine block of motor nerve	Following section of motor nerve	
34	Bilateral	7	Dial	Sciát. Yes	A.Ch.	jugul.	3	Marked		Marked	5
36	Bilateral	9	Dial	Fem. Yes	A.Ch.	jugul.	4	Marked		Marked	7
47	Bilateral	8	Dial	Sciat. Yes	A.Ch.	ilíac.	10	Marked		Moderate	8
51	Bilateral	14	Dial	Fem. No	A.Ch.	renal	10	Slight		Moderate	
54	Bilateral	?	Dial	Fem. Yes	A.Ch.	renal	3	Moderate	Marked	Marked	
60	Bilateral	8	Dial	Fem. Yes	A.Ch.	renal	3	Moderate	Marked	Marked	6
52	Bilateral	27	Dial	Fem. Yes	A.Ch.	renal	1	Moderate	Marked	Marked	
52	Bilateral	27	Dial	Fem. Yes	K.	renal	50	Absent		Moderate	
49	Bilateral	9	Dial	Fem. No	K.	renal	150	Slight		Moderate	
55	Bilateral	18	Dial	Fem. Yes	K.	renal	25	Slight	Marked	Marked	
42	Bilateral	13	Dial	Sciat. No	A.Ch.	femor.	4·80	Moderate		Doubtful	
35	Left	5	Decerebr.	Sciat. Yes	A.Ch.	jugul.	1	Moderate		Abolished	
11	Left	15	Decerebr.	Sciát. No	A.Ch.	jugul.	10	Absent		Absent	

when there is a previous hypersensitivity to acetylcholine of the neuromuscular synapsis, obtained by diverse procedures as, for example, spinal hemisection, lesion of the cerebellum and brain stem, tenotomy, occlusion of the thoracic aorta, and particularly medullary pyramidotomy.

The acetylcholine and potassium sensitization occurring immediately after nerve section is irreversible, is different to the postetanic effect, appears to be more evident when the motor nerve of antagonistic muscles of the explored muscle has been previously sectioned and is also observed in adrenalectomized and sympathectomized animals. It must be noted that all our experiments have been carried out on animals anesthetized with Dial or chloral-morphine with the brain otherwise intact, with the exception of lesions previously effected in chronic experiments.

It is well to remember that in most of our experiments, the acetylcholine injections were given in the lower part of the abdominal aorta through the renal artery, and that in such a way the drug reached the neuromuscular synapsis and not the spinal cord. Thus the possible effect of the acetylcholine on the spinal cord is dismissed.

The type of myographic curves obtained suggests that responses were due to the addition of the contraction and the contracture. It has, however, not been investigated whether the responses were accompanied by action potentials in the muscle.

This series of facts leads to the conclusion that acetylcholine and potassium hypersensitivity immediately after motor nerve section is due to a sudden and permanent change in the condition of the neuromuscular synapsis as a result of motor nerve interruption.

This phenomenon had already been suggested by McIntyre (1947), who, so as to explain the rhythmic muscle activity after denervation, which is evident some days after neurotomy, advanced the idea that the presence of the normal inactive motor nerve keeps the muscle at rest. The same author, together with Bennett (1949) has recently observed that "*shortly* after nerve section the response of the muscle to direct stimulation through the distal portion of the nerve is *somewhat enhanced*, as is the response of the muscle to intraarterial injection of acetylcholine".

This phenomenon, however, has not received the necessary attention. McIntyre and Bennett mention it only to support their hypothesis of an inhibitory effect on the intact motor nerve, an inhibitory action which is undoubtedly present in some lower species, as it was pointed out before, but which is not confirmed in the mammals. In this paper we have only tried to show that interruption of the connections between the neuromuscular synapsis and muscle, on one side, and the central nervous system, on the other, shows an immediate increase of sensitivity to acetylcholine or potassium, and that this irreversible phenomenon is due exclusively to this interruption and not to any foreign facts.

Analyzing some of the facts found in our experiments makes us believe that a relative integrity of the central nervous system is necessary. In fact, the phenomenon was not observed in the decerebrated animals or animals with large spinal cord lesions. Cannon and Haimovici (1939), when studying the sensitization of spinal motoneurons by partial

denervation, observed that if the motor nerve was intact the muscular response to injection of acetylcholine and other drugs in the thoracic aorta, were the result of the addition of the effect of this drug on the spinal motoneurons and on the neuromuscular synapsis, and that the response was less complicated and of less amplitude when the motor nerve had previously been sectioned; but these authors sectioned the spinal cord at the level of the foramen magnum before the acute experiment. Thus everything seems to indicate that the differences between Cannon and Haimovici's studies and ours, reside in the integrity of the encephalo-spinal connections, in the acute experiments.

At the present stage of our experiments it can be said, that interruption of the motor nerve produces an immediate hypersensitivity of the neuromuscular synapsis and the muscle to the injection of acetylcholine or potassium into the blood stream, which could depend on an inhibitory effect of the motor nerve, and can, in the acute experiment, only be seen when the central nervous system is relatively intact. Although from the present studies some conclusions could be drawn as to the long systems which take place in this phenomenon, such analysis should be subject to further investigation.

SUMMARY

In more than 50 cats grouped in the following manner: normal, pyramidotomized, spinal hemisections, lesions of the cerebellum, and with sectioned gastrocnemius tendon; and in 5 dogs with paraplegia from anemia of the thoracic spinal cord, it was found that the intact motor nerve of the gastrocnemius or quadriceps muscle, exercised an inhibitory action on the acetylcholine or potassium response of the muscle. This effect was slight in the normal animals in which a great amplification was necessary for its demonstration, but it was clearly apparent in the animals sensitized by the mentioned lesions. The comparison was shown measuring the response obtained after sectioning the nerve or blocking it with procaine, injecting the acetylcholine or potassium into the bloodstream, usually through the renal artery. When the nerve was blocked, the inhibitory action reappeared when the anesthetic block ceased.

The interpretations accorded to this phenomenon are discussed. It is pointed out that it appears even when the adrenals and lumbar sympathetic chain are removed, that it is different to the postetanic effect, because of its permanency and because it appears without provoking a previous muscle contraction, and that it needs a certain integrity of the central nervous system.

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ANTIDIURETIC ACTION OF HUMAN PLASMA

H. CROXATTO, F. ANDRADE AND L. BARNAFI

(Laboratorio de Fisiología, Universidad Católica de Chile, Santiago, Chile)

IT has been assumed that the normal blood contains an antidiuretic factor and it has been thought that this factor is identical with vasopressin (see the work of Marx and Schneider, 1934; Melville 1937; Birnie *et al.*, 1949; Dicker and Grinsburg, 1950). Results were sometimes contradictory though indeed only apparently. Thus Walker (1939) and Hare *et al.* (1941) did not find the antidiuretic factor in the blood of dogs; but Ames *et al.* (1950) were able to obtain an antidiuretic effect with the blood of the jugular vein of the dog after intense stimulation of the osmoreceptors. There were also contradictory statements concerning the antidiuretic factor in the blood of eclamptic women. Thus, Anselmino *et al.* (1931) have reported hypertensive and antidiuretic properties of the blood obtained from such patients; but other have not confirmed these results (Hurwitz and Bullock, 1935; Byrom and Wilson, 1934; Lewitt, 1936; Blaszo and Dubransky, 1940).

These contradictory statements and the great variability of some of our own preliminary results led us also to think that variations might be conditioned by the experimental procedures used. Therefore one of the objects of the present work was to study those circunstances which might influence experimental results such as (1) temperature; (2) hemolysis; (3) coagulation; (4) plasmatic enzymes that are presumably activated after the withdrawal of the blood (fibrinolysin); (5) dialysis through cellophane membranes; (6) sulfhydril substances; (7) bacterial contamination. Another very interesting problem sprung up in the course of this investigation when two unexpected but independents facts were observed: first, both plasma and serum lost, either partially or completely, their antidiuretic properties if dialyzed for a long time; and secondly, an antidiuretic factor was obtained by hydrolysis of plasma globulins with pepsin (Croxatto, Rojas and Barnafi, 1951). It was then but natural to

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study the question whether proteins of previously dialyzed plasma were still capable of forming the antidiuretic factor when incubated with pepsin.

METHODS

Both plasma and serum of normal individuals were examined. One hundred and seventeen samples were analyzed without previous control of the water intake. Also three cases of diabetes insipidus were studied. In experiments in which peptic digestion was performed, 0.5 mg. of crystalyzed pepsin per 10 ml. of either plasma or serum was added. The sample was maintained at pH 2.5 during 4 hours at 37° C. When incubation or dialysis lasted over 2 hours toluol (1 to 3 %) was added except if indicated otherwise.

TABLE I

Antidiuretic activity of serum of normal humans, injected into rats (0.5 to 1.5 ml of serum per 100 g body weight) to whom water has been administered. (Burn's Test)

Number of samples	% of water excreted *		
	60 min	90 min	120 min
77	8.1 ± 1.2	13 ± 1.8	19 ± 2.1

* With 0.9 % NaCl, 50 % of the administered water was excreted at 64 minutes.

Burn's test (1931) was used for measuring the antidiuretic effect. Each sample was tested in 3 or 4 rats. The volume injected ranged from 0.5 to 2 ml. of either serum or plasma per 100 gm. of rat. Each experiment was accompanied by a control group of rats injected with 0.9 % NaCl, In some experiments the vasopressor action of the respective sample also was studied. Blood pressure was recorded from the carotid artery of both cats and rats anesthetized with Dial, up to 4 hours after the intraperitoneal injections of the sample.

RESULTS

Antidiuretic activity was expressed in percentage of administered water excreted after 60, 90 and 120 minutes. As seen in Tables I and II (before dialysis) and in figs. 1 A, 2 A, 3 C, 4 A and 5 A, injection of

either plasma or serum (from 0.5 to 1.5 ml. per 100 gm. of body weight) induced a clear cut antidiuretic effect compared to control groups receiving 0.9 % NaCl (fig. 3 B and 4 D) in which excretion was about 50 % at 64 ± 4.1 minutes (Almeyda, 1951).

In spite of individual variations it was fully evident that the antidiuretic effect increased, both with serum and plasma, the more, the greater

TABLE II

Antidiuretic activity of fresh plasma and serum of normal humans before and after dialysis, injected into rats (0.5 to 1.5 ml. per 100 g body weight) (Burn's Test)

Number of samples	BEFORE DIALYSIS % of water excreted *			AFTER DIALYSIS % of water excreted *		
	60 min	90 min.	120 min.	60 min.	90 min.	120 min.
40	5.3 ± 1.5	9.6 ± 2.0	16 ± 6.3	40 ± 3.1	57 ± 3.4	66 ± 3.7

* With 0.9 % NaCl, 50 % of the administered water was excreted at 64 minutes.

the quantities given were. With 0.4 to 0.7 ml. per 100 gm. the average excretion after 120 minutes was of 35 %; with 0.8 ml. 27 % was excreted, and with 0.9 to 1.5 ml only 17 %. These values were obtained with samples taken 30 minutes before the experiment, or 12 hours previously but kept at 0° C.

1) *Effect of temperature.* — The antidiuretic activity of fresh plasma centrifugated at low temperature 30 minutes after it was obtained and then kept at 0° C was compared to plasma incubated at 37° from 2 to 24 hours. Fig. 1 and 2 shows that prolonged incubation destroyed the antidiuretic potency of plasma (compare A to B and C of fig. 1; A to B, C and D of fig. 2). One may presume that this was due to enzymes present in the plasma.

2) *Effect of hemolysis.* — When either serum or plasma were maintained at 0° C, no significant loss of antidiuretic activity occurred. On the contrary, when 1 ml. of a suspension of hemolyzed red cells was added to 10 ml of plasma and the latter was kept at 0° C it gradually lost its antidiuretic action. Loss was accelerated if the sample was kept at 37° C (fig. 1, compare B' to B, and C' to C).

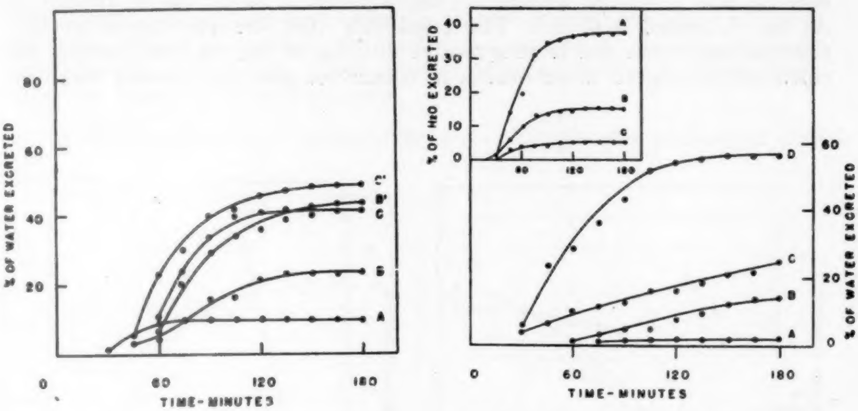


FIG. 1.—*Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Intraperitoneal injections of ml per 100 gm. body weight: A. 1.2 ml. of human plasma kept at 0° C. up to the moment of the injection. B. 1.2 ml. of human plasma incubated during 4 hours at 37° C. with toluol. C. 1.2 ml. of human plasma incubated during 12 hours at 37° C. with toluol. B'. 1.2 ml. of human plasma incubated during 4 hours at 37° C. to which 1 ml. of a hemolytic solution per 10 ml. of plasma and toluol was added. C'. 1.2 ml. as B' but incubated during 12 hours.*

FIG. 2.—*Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Intraperitoneal injections of ml per 100 gm. of body weight: A. 1.3 ml. of human plasma kept at 0° up to the moment of the injection. B. 1.3 ml. of plasma incubated at 37° C during 4 hours with toluol. C. 1.3 ml. of plasma incubated at 37° C during 8 hours with toluol. D. 1.3 ml. of plasma incubated at 37° C during 12 hours with toluol.*

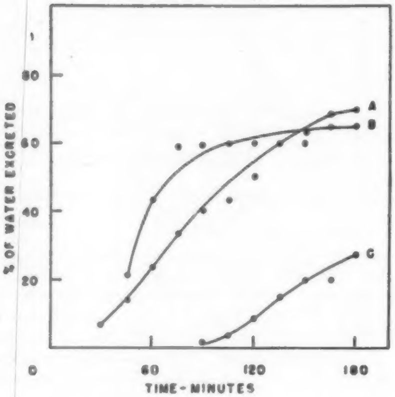
Insert: A. 1.3 ml. of serum incubated at 37° during 4 hours, with toluol. B. 1.3 ml. of serum incubated at 37° C during 4 hours, after being hydrolyzed with 1 mg. of pepsin per 1 ml. at pH 2.5 during 4 hours. C. 1.3 ml. of human serum kept at 0° C up to the moment of the injection.

3) *Effect of coagulation.* — There was no difference in antidiuretic activity between citrated plasma and blood serum obtained either from spontaneous coagulation or by the addition of trombin (5 U per ml). This observation suggests that enzymes conditioning either coagulation or formation of fibrin do not interfere in antidiuretic action. Our finding does not agree with that of Dicker and Grinsburg (1950).

4) *Effect of fibrinolysin.* — Addition of 1 mg of purified and lyophilized fibrinolysin to 1 ml of dialyzed or non-dialyzed plasma or serum had no influence on antidiuretic activity. Neither was the antidiuretic activity diminished when concentrated solutions of fibrinogen were incubated with fibrinolysin.

5) *Effect of dialysis.* — Dialysis both of plasma and serum was performed in cellophane tubes for 12 hours against either tap water or distilled water. A progressive decrease and even exhaustion of antidiuretic

activity was produced (Table II; fig. 3 comp. A to C; fig. 4 comp. C to A; fig. 5, comp. B to A). The possibility that the diminished antidiuretic potency were due to progressive dilution of the dialyzed sample was ruled out by control experiments in which non-dialyzed plasma was dilu-



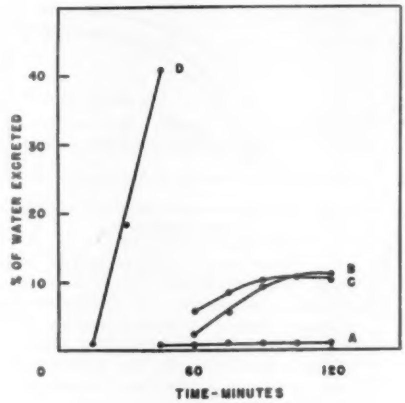


FIG. 3. — *Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Inraperitoneal injections of ml. per 100 gm. of body weight: A. 1.3 ml. of fresh serum dialyzed during 24 hours against tap water. B. 1.2 ml. of 0.9 % NaCl. C. 1.2 ml. of human serum kept at 0° C during 24 hours.*

FIG. 4. — *Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Intraperitoneal injections of ml. per 100 gm. of body weight: A. 1.3 ml. of plasma kept at 0° C up to the moment of the injection. B. 1.3 ml. of the external solution of dialysis equivalent to 25 % of the total after concentration by Grollman's method. At 60 min. the antidiuretic effect of the external solution was even more considerable than that of the dialyzed plasma though only part of the plasma has been injected, and though as seen from C the antidiuretic effect of the plasma was much less diminished as for instance in fig. 3 A. C. 1.4 ml. of dialyzed human plasma during 24 hours against distilled water. D. 1.3 ml. of* J.T *% NaCl.*

ted in the same proportion. That the decrease was due to the passage of the active factor through the cellophane membrane was demonstrated by the fact that it was possible to obtain from the water against which dialysis was performed a substance producing an antidiuretic effect (fig. 4). For obtaining and preserving this substance the following technique was used. The pH of the distilled water was adjusted to 4.5; carbon Norit was added as an adsorbent. After several hours of dialysis under constant agitation the carbon was recuperated through filtration. After drying it was treated with glacial acetic acid according to the method described by Grollman and Woods (1949).

6) *Effect of glutathione.* — Loss of antidiuretic potency was accelerated when glutathione was added to plasma in the proportion of 5 mg. per 1 ml. It is known from the work of Croxatto *et al.* (see Céspedes and Croxatto, 1949; Daza, 1950) that glutathione favors also the inactivation of vasopressin and oxytocin.

7) *Effect of bacterial contamination.* — Antidiuretic potency of plas-

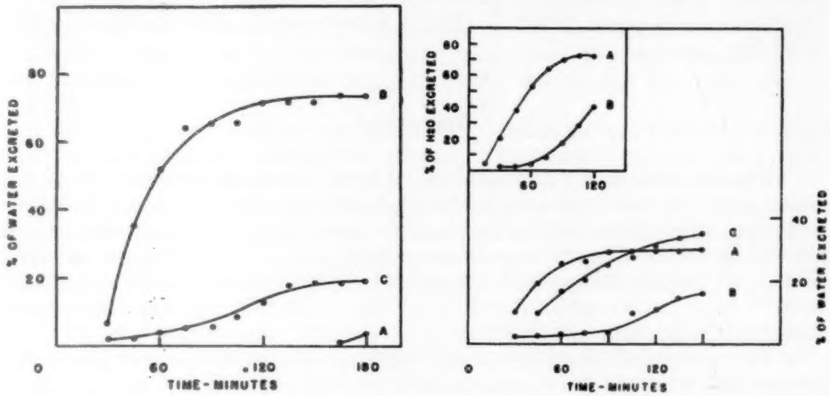


FIG. 5. — *Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Intraperitoneal injections of ml per 100 gm. of body weight: A. 1.3 ml. of human serum kept at 0° C during 24 hours. B. 1.4 ml. of human serum, dialyzed during 24 hours against tap water. C. 1.4 ml. of human serum dialyzed during 24 hours and then incubated at 37° during 4 hours with 1 mg. of pepsin at pH 2.5.*

FIG. 6. — *Each curve represents the percentage of water excreted by 4 hyperhydrated rats. Intraperitoneal injections of ml per 100 gm. of body weight: A. 1 ml. of human plasma kept during 8 days at 0° C up to the moment of the injection. B. 1 ml. of plasma incubated during 20 hours at 37° C without the addition of toluol. C. 1 ml. of plasma incubated during 20 hours at 37° C with toluol.*

Insert: A. 1.2 ml. of human plasma dialyzed during 24 hours against tap water. B. 1.2 ml. of human plasma dialyzed during 24 hours and then incubated during 12 hours with staphylococcus aureus: passed through a Seitz filter before the injection.

ma either dialyzed or incubated at 37° C, considerably increased when precautions to prevent bacterial contamination and growth were not taken (fig. 6, comp. C to B). When a sample of plasma was first incubated at 37° C during 12 hours with a culture of staphylococcus and then sterilized, before injection, by filtration through a Seitz filter (fig. 6, insert), the filtrated product (B) showed intense antidiuretic effect compared to the control sample (A).

8) *Effect of digestion with pepsin.* — Exhaustion of antidiuretic activity through dialysis suggests that the antidiuretic substance is of

small molecular weight. The phenomenon of exhaustion offers also the possibility of a new approach to the study of the antidiuretic factor. We have shown in former work that an antidiuretic factor can be obtained through digestion of hypertensinogen with pepsin (Croxatto, Rojas and Barnafi, 1951 a and b). On the basis of all these findings we thought that it would be of considerable interest to examine the question whether dialyzed plasma which was almost void of any antidiuretic activity would recover it when digested with pepsin (pH 2.5 at 37° C. during 4 hours). Positive results were obtained (fig. 2, insert, comp. B to A; fig. 5, comp. C to B).

DISCUSSION

The reported experiments give evidence that either serum or plasma contain one or more factors which counteract excretion of water in hyperhydrated rats. Our findings also show how important and even fundamental are the different procedures employed in the treatment of either plasma or serum, when studying its antidiuretic potency. This may contribute to a better understanding of the contradictory results reported in this field by other authors.

The possibility that the antidiuretic effect might be due in our experimental animals, to a non-specific protein shock has been ruled out. Intraperitoneal injection of either plasma or serum does not modify blood pressure in the carotid artery of rats. Furthermore, the decrease of the antidiuretic potency of plasma after dialysis or incubation at 37° C with toluol indicates that the antidiuretic effect is not produced by proteins but by a substance or substances which are both dialyzable and unstable. Neither can the diminution of the antidiuretic potency of dialyzed plasma be explained by a diminution of the saline concentration: maintenance of the saline concentration and osmotic pressure at a normal level, by adding NaCl, did not protect the dialyzed plasma against loss of its antidiuretic potency.

Our results and especially those with dialyzed plasma suggest that the antidiuretic potency of plasma is dependent on two different factors, or two different stages of the same factor, one free and the other partially or completely bound to some protein. The latter factor is not dialyzable and is, apparently, liberated only by enzymatic action. One hydrolytic enzyme known to be present in normal blood is of an especial interest here. We refer to fibrinolysin. Its effect varies greatly from one individual to another and one may wonder whether individual variations of the antidiuretic potency of plasma or serum might be due to fibrinolysin. However, our results with fibrinolysin show that this enzyme is unable to liberate the antidiuretic factor.

The results reported in this paper do not allow to discuss the question whether the antidiuretic action of plasma is dependent on the secretory activity of the hypophysis. However, the statement seems important that serum obtained from three cases of diabetes insipidus showed normal antidiuretic activity. If this disease is due to a failure of the

hypophysis to secrete vasopressin as generally assumed, the antidiuretic factor of the plasma would not be related to this hypophysial hormone. This problem could be attacked by studying the antidiuretic activity of the blood of hypophysectomized animals.

Summary

1) Diuresis produced in rats by administration of water (5 % body weight; test of Burn) was counteracted by the intraperitoneal injection of either plasma or serum obtained from recently centrifugated blood of humans. The quantities injected were 0.8 to 1.5 ml. per 100 gm. of rat.

2) Prolonged incubation at 37° C, hemolysis or additon of glutathione decreased the antidiuretic potency of plasma or serum; coagulation or addition of fibrinolysin did not influence the antidiuretic potency.

3) Bacterial contamination increased the antidiuretic potency.

4) Consequently, individual variations of the antidiuretic potency of plasma or serum can be avoided to a certain degree when precautions are taken such as rapid cooling, prevention of hemolysis and limited storage time.

5) The antidiuretic potency was decreased by dialysis of plasma or serum through a cellophane membrane. Similar to incubation at 37° C dialysis caused in some experiments even complete exhaustion of the antidiuretic potency.

6) Diminution of antidiuretic potency by dialysis was not due to dilution but to the passage of an antidiuretic substance into the water in which dialysis was performed. An antidiuretic substance was obtained from the distilled water used during dialysis with the help of an adsorbent (Norit).

7) Plasma which has lost its antidiuretic potency due to incubation or dialysis may regain it if digested with pepsin at pH 2.5 during 4 hours.

8) From the experimental findings mentioned above the conclusion is drawn that the antidiuretic potency of the blood plasma is due to a substance (or substances) which is destroyed by some enzyme present in the plasma or serum; that the inactivation of this substance is accelerated by incubation at 37° C., by hemolysis and by the addition of glutathione; that the antidiuretic substance dialyzes through cellophane membranes; and that the antidiuretic substance is present in the plasma partly free and partly bound to proteins from which it can be freed through enzymatic action.

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ACTION OF BANTHINE ON THE MOTILITY OF THE SMALL INTESTINE CONTROLLED BY THE RAPID METHOD

JUAN NASIO

(Servicio de Gastroenterología. Hospital "Clemente Alvarez". Rosario, República Argentina)

WE have corroborated clinically and experimentally [11, 12] that Banthine has a depressing effect on gastrointestinal motility. Longino, Grimson *et al* [10] examined radiologically the gastrointestinal tract of seven dogs under the action of Banthine and proved that an injection of Banthine given 10 minutes before barium sulphate is administered, may delay considerably gastric evacuation and the passage of opaque food through the rest of the intestine.

Studying the human digestive tract with the intragastrical balloon, Longino *et al* [9] found that a parenteral dose of 0.1 to 0.4 mg/kg of Banthine caused the immediate cessation of gastric contractions in all cases.

Walters *et al* [15] also found that within 20-30 minutes following 100 mg of Banthine orally, gastric contractions ceased and remained absent for approximately two hours. Within fifteen minutes this drug caused a depression in the intestine's motor activity which lasted two hours.

Chapman *et al* [2] studied gastrointestinal contractility under the action of Banthine by means of their multiple balloon kymographic recordings. Within 15-30 minutes of administering 100 mg of Banthine the contractile waves of the stomach decreased or ceased.

Examining comparatively the antispasmodic action of different quaternary amines on the human colon, Kern *et al* [7] found that Banthine was more effective than atropine.

Hambourger *et al* [5] studied the pharmacological action of Banthine, and although its activity was found to be equivalent to 2/3 of that of atropine, there are two important facts to be considered: a) In rabbits it relaxes the ileum spasm caused by acetylcholine. b) In dogs and cats

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Investigations related to the blockading action of some drugs on man's small intestine have proved that intestinal motility is considerably reduced by certain anticholinergic drugs. Holt *et al* ([6]) found that tetraethylammonium, which blockades the autonomic ganglions, stops intestinal movements. Bone and Bruce Crow ([1]) studied the action of dibutoline on man's stomach and small intestine, from the radiological point of view. For that purpose they administered several subcutaneous doses of 10-25 mg of this drug and found that the motility of stomach and small intestine were considerably disturbed. In the stomach there was a marked decrease of peristaltic activity, but it was not abolished. It also decreased peristaltism in the intestine and prolonged the opaque column's transit without altering the mucose shadows. The action of dibutoline is inmediate but precarious, the colateral reactions are scarce and it does not act if administered orally. Considering that it has been proved in the above mentioned studies that anticholinergic drugs act on gastrointestinal motility when administered parenterally, we decided to investigate the action of Banthine on man's small intestine given orally, having in mind Lepore's *et al.* ([8]) work on the subject.

METHODS

We have met a number of obstacles in our investigation, due to the difficulties which arise in the practice of ordinary radiology of the small intestine. Several methods have been used to study this organ: Pendergrass ([13]) used barium and distilled water; Golden ([4]) saline solution; Pesquera ([14]) preconizes the enema of the small intestine, that other authors put in practice by means of Wilson-Sawyer's probe. All these methods require numerous plates, every half hour during 6 to 9 hours, a continuous radioscopic observation, great loss of time for patient and radiologist, without considering the dangers implied by the radiotions if the tolerable limits are surpassed; and the cost of the great amount of plates that must be taken, as Garcia Capurro has observed ([3]). Confronted with these difficulties we chose the rapid method for studying the small intestine, presented by Weintraub and Williams ([16]) in 1949. Weintraub observed in 1941 the importance of cold drinks on the intestinal transit's acceleration. Weltz ([17]) duly pointed out this observation showing that, while barium mixed with hot water delayed the small intestine's motility, it accelerated this movement when mixed with ice. However we are indebted to northamerican authors for establishing a method which we consider of great importance for the radiological diagnosis of this neglected organ. This neglect has not been due to the absence of an important pathology but to the lack of a practical, rapid and simple method of diagnosis as the one here considered. While through the rapid method the cecum can be filled in half an hour or less in over 80 % of the cases, through the classic methods the opaque food only reaches this organ after an hour, and this in less than 50 % of the cases.

Our study of the action of Banthine on the small intestine was performed in the following way:

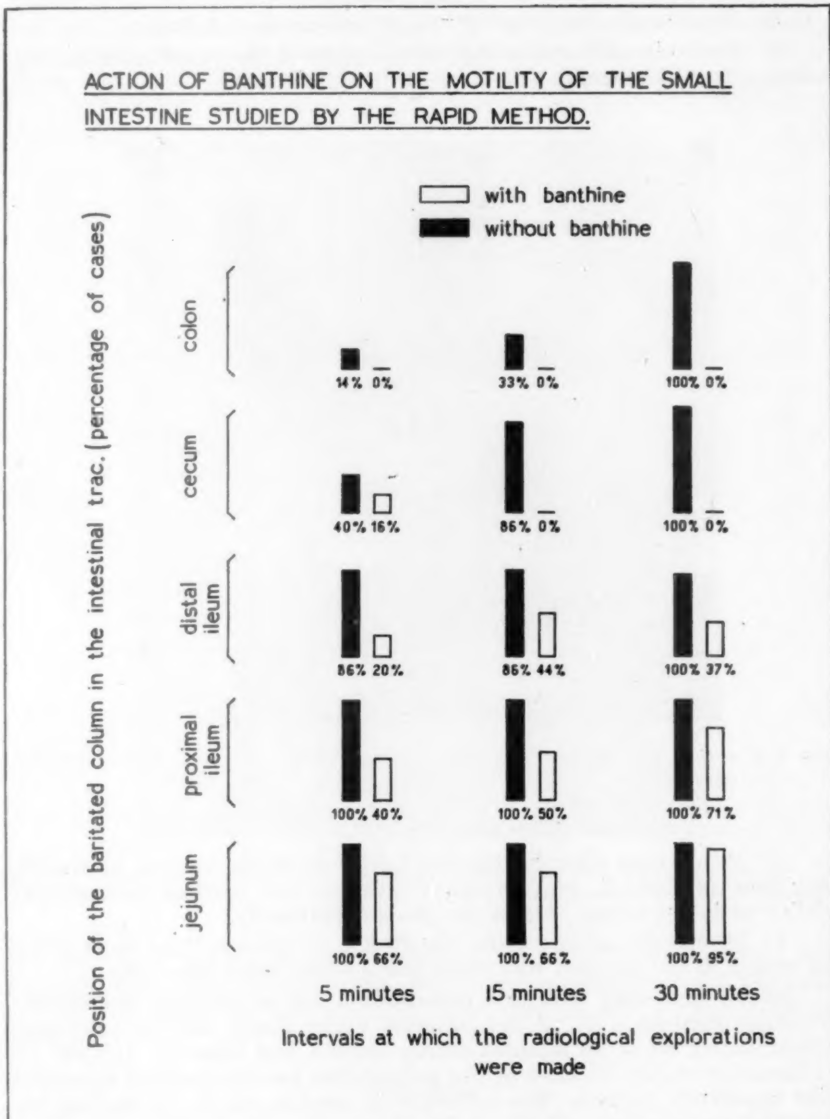


FIG. 1.—*Results of the examinations of the small intestine by the rapid method, with and without Banthine.*

1) The fasting patient drank 120 ml. of barium sulphate with 120 ml. of isotonic saline solution at room temperature.

2) Immediately he drank 230 ml. of cold saline solution.

3) 5 minutes after drinking this, a plate of the small intestine was taken (14 x 17 inches).

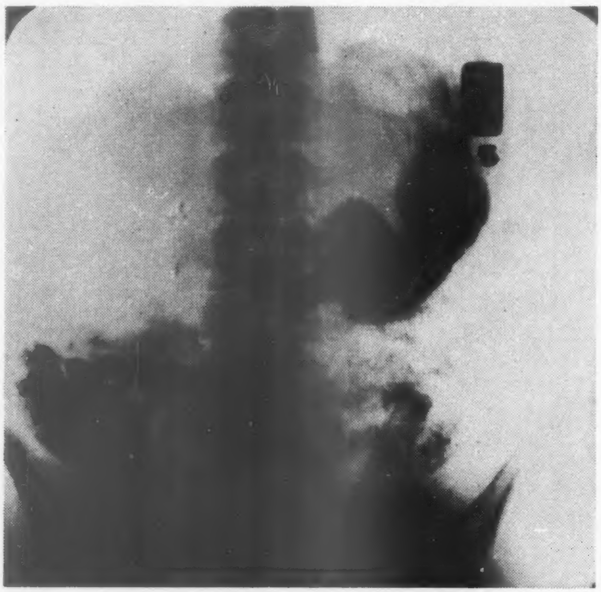


FIG. 2. A. — *Case 6. Intestinal tuberculosis. Plate taken after 15 minutes. No Banthine administered; ascending colon filled.*

4) 15 minutes after having the first cold drink, he was given another dose of 230 ml. of cold saline solution and another radiographic plate was taken (equal size to the above mentioned).

5) 30 minutes after having the first cold drink a third dose of 230 ml of cold saline solution was given and another plate was taken.

Seven days after this first examination was performed, we followed the same method with all the patients under study, having previously administered, 30 or 40 minutes before barium was ingested, 150-200 mg of Banthine orally. In most of the patients the gastrointestinal apparatus was apparently normal. The influence of psychic, physic or feeding factors that could interfere in the control of this investigation were foreseen. In the presence of allergic food, an unusual rise of temperature, or a great anxiety in the patient, the examination was rejected and another was performed in the most natural conditions possible.

Twenty patients were used for our investigations; seventeen had apparently no lesions of the small intestine, two were gastrectomized and one had intestinal tuberculosis. Eight of them were examined radiologically three times with banthine and three without, at 5, 15 and 30 minutes after the treatment was started. In the other cases, we examined

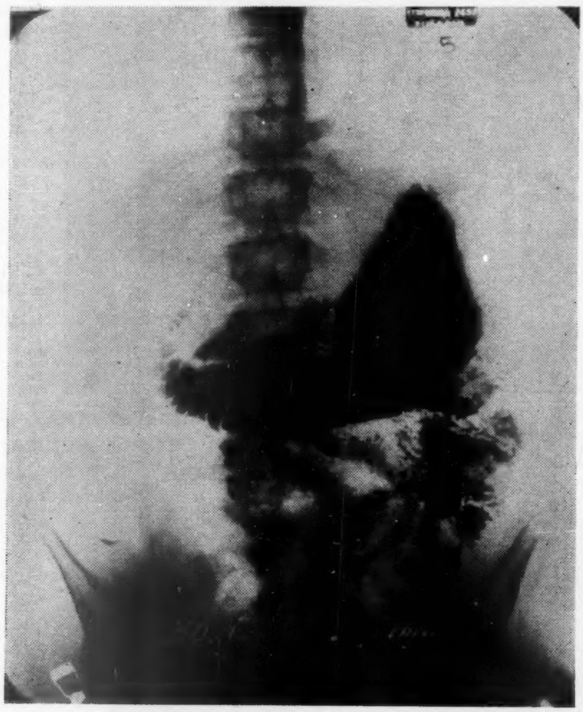
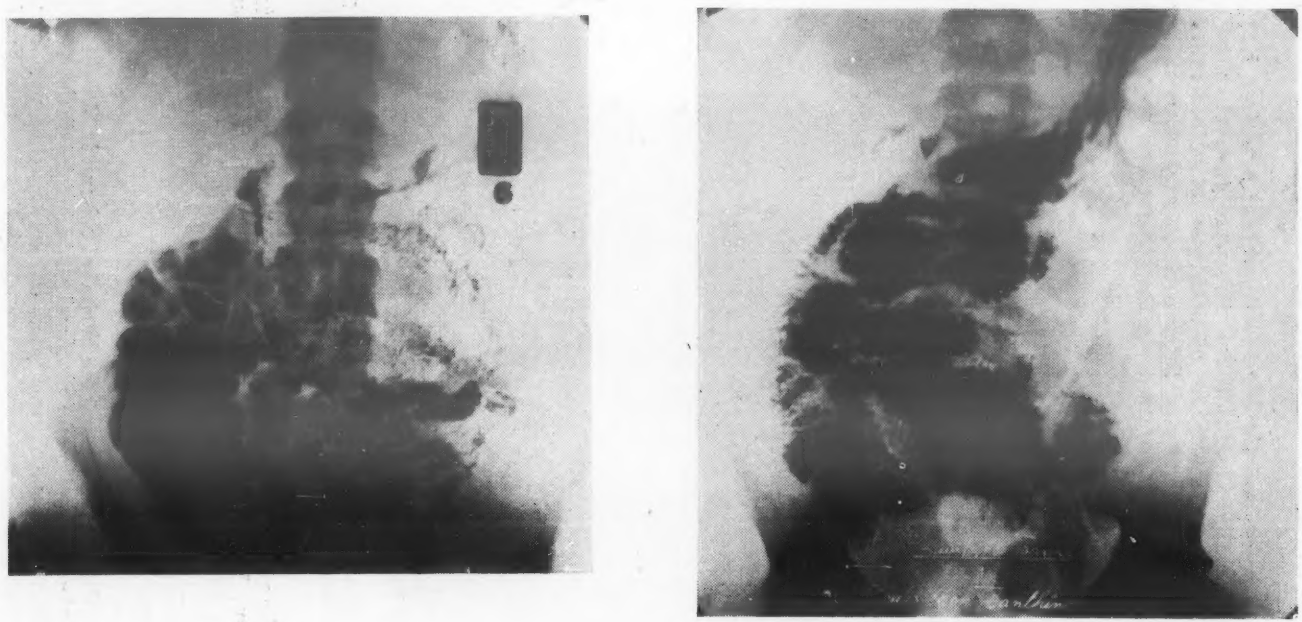


FIG. 2. B.—*Plate taken after 30 minutes. Banthine previously administered. The opaque material has not reached the colon. Greater stasis of the small intestine.*

the patients by the rapid method, taking plates only 30 minutes after starting the treatment. We also made radiological explorations with the usual methods to some of these patients; these results will be published separately.

RESULTS

Fig. 1 shows the progression of the baritated column in the different portions of the intestine; jejunum, proximal ileum, distal ileum and cecum. If the column has gone further than the cecum, that part of the intestine is mentioned as the colon. The examination of the radiographic



A B

FIG. 3.—*Case 8. Normal. A: Plate taken after 30 minutes. No Banthine administered. Right colon filled; stomach empty. B: Plate taken after 30 minutes. Banthine previously administered. The opaque material has not reached the cecum. Incomplete gastric evacuation and stasis of small intestine's loops.*

plates studied at different time periods, with or without Banthine gave the following results:

A) In the 5 minutes plate, opaque food had reached the proximal ileum in 100 % of cases and the distal ileum in 86 %; under the action of Banthine it had reached there only in 40 % and 20 % of the cases respectively;

B) While the baritated columun in 6 % and 100 % of the cases filled the cecum in 15 and 30 minutes, under the action of Banthine it never reached this organ. (Fig. 2 and 3);

C) The maximum intestinal progression of the baritated column under action of Banthine after 30 minutes was the distal ileum, which it reached only in 37 % of the cases.

D) The portions of the small intestine that were filled in the majority of the cases were the jejunum and the proximal ileum (95 % and 71 % of the cases respectively).

According to these results, Banthine reduces considerably the small intestine's motility to an average of 60 % in 30 minutes. We corroborated in 20 cases by this rapid method the conclusions to which Lepore *et al* ([18]) had arrived through the classic method, or Golden's method in seven cases.

Summary

1º) Oral administration of 150-200 mg of Banthine reduces considerably the motility of man's small intestine, controlled by the rapid method of Weintraub and Williams.

2º) While the baritated column filled the cecum in 15 and 30 minutes in 86 % and 100 % of the cases, it never reached this organ under the action of Banthine in the same period of time.

3º) The maximum intestinal progression of the baritated column under action of Banthine after 30 minutes was the distal ileum and this only in 37 % of the cases.

4º) The average reduction of motility of the baritated column was of 60 % in 30 minutes.

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EFFECT OF RENIN ON DIURESIS

H. Croxatto, L. Barnafi and J. Passi

(Laboratorio de Fisiología. Universidad Católica de Chile. Santiago, Chile)

Pickering *et al* (1940) Hughes-Jones *et al* (1949) demonstrated that in rabbits an intense stimulation of diuresis is produced by the intravenous administration of renin obtained from the kidneys of animals of the same species. A similar effect has been observed also under other experimental conditions. Thus Brandt *et al.* (1948) have shown that diuresis is greatly stimulated in hyperhydrated rabbits with renin obtained from the pig, and Addis *et al.* (1949) and Croxatto *et al.* (1951) found that diuresis is stimulated after the intraperitoneal injection of renin in rats with free water intake. Masson *et al.* (1950) reported the same effect in unilaterally nephrectomized rats fed with an excess of NaCl and receiving renin by subcutaneous injection, and Sellers *et al.* (1951) in hyperhydrated rats with the intraperitoneal administration of renin from the pig.

We thought of interest studying the diuretic effect of renin systematically by considering separately a number of variables which seemed of importance.

Methods

1.—*Renin and hypertensin used.*—The solutions of renin were prepared from kidneys of human, pigs and rats according to the technique described by Braun-Menendez *et al.* (1943) and Dexter *et al.* (1943). The dry weight of these preparations was of the order of 0.6 to 6 g %. The pressor activity of renin was controlled by intravenous injection in cats and rats. Isotonic solution were obtained by adding 0.7 % of NaCl. The better purified extracts contained 25 U. per ml (Braun-Menendez *et al.*). Usually 0.5 to 1 ml per 100 g of body weight was injected. Unexpectedly "inactive" renin was obtained from kidneys of pigs treated ac-

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cording to the method of Dexter *et al.* It was considered inactive when not presenting any hypertensive action and not forming hypertensin during incubation with hypertensinogen. The solution of hypertensin was prepared according to the technique of Braun-Menendez *et al.* It contained 30 U. per ml, and 1.5 to 9 U per 100 g body weight were given daily.

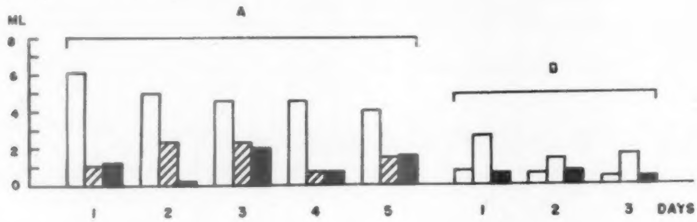


FIG 1.—*Urine volume expressed in ml. per 100 g body weight during 12 hours. Each column represents the average for 6 rats which drank tap water. A. White columns: Rats which received daily injections of 0,25 ml. of renin obtained from pigs, given intraperitonealy. Stripped columns: This group received the same dose of renin but given subcutaneously. Black columns: Rats injected with 0.9% NaCl intraperitoneally. B. Urinary excretion 40 days after the beginning of the experiment. White columns: Intraperitoneal injection of renin from pigs. Black columns: 0.9 % NaCl given intraperitoneally.*

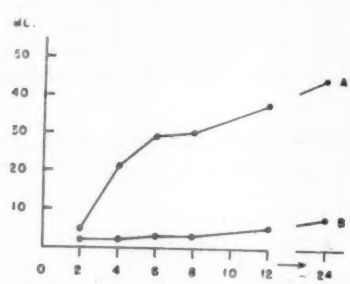


FIG. 2.—*Urinary volume during 24 hours. A. Group of 6 rats which received 0.5 ml. of renin per 100 g of body weight, given intraperitoneally. B. Control group of 6 rats injected with 0.9 % NaCl given intraperitoneally.*

2. — *Animals employed.* — There were two series of animals: first, rats which had free water intake, and secondly, rats which were forcibly hyperhydrated.

The animals of the *first* series —160 adult rats of both sexes of 200 to 300 g— were divided into groups of 3 or 4 rats. Both water intake and urinary excretion were measured in metabolic cages from the mo-

ment of injection up to 12 hours afterwards. During this period of time no food was given. The influence of renin on water intake and on urinary excretion was followed up to the 10th or the 40th day. Renin was injected every 24 hours, except for 2 or 3 days, but without interrupting measurement of water intake and urinary excretion.

The animals of the *second series* —140 adults rats of both sexes— were placed under conditions as required by the test of Burn. They received no food during 24 hours but were allowed free water intake. On the next day water was administered through a catheter; the amount given

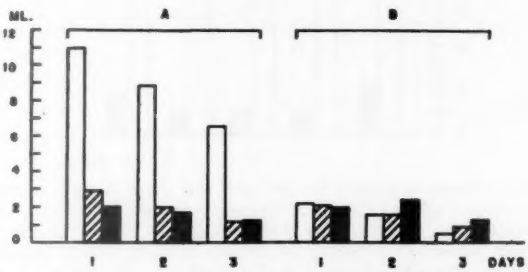


FIG. 3.—*A. Urinary volume expressed in ml. per 100 g. and 12 hours. Each column is the average of 6 rats which drank tap water. White columns: Rats which received a daily injection of 0.5 ml. of renin given intraperitoneally. Stripped columns: This group received the same dose of renin subcutaneously. Black columns: Control group which received an intraperitoneal injection of 0.9 % NaCl. B. This group was placed under conditions similar to those of group A, but received equal doses of "inactive" renin.*

was equal to 5 % of the body weight. In this series again the various groups of 3 or 4 animals were placed in metabolic cages.

3.—*Variables studied.*—The following seven variables of the diuretic action were examined in the two series mentioned: the influence of the way of administration —intraperitoneal or subcutaneous— of the solutions of renin; the influence of preceding treatment with renin on the response to renin; the comparative action of renin obtained from different animals; the comparative diuretic action of solutions containing renin and of "inactive" solutions; the influence of NaCl; the influence of hypertensin injected by different routes, and the influence of adrenalectomy.

RESULTS.

Renin given intraperitoneally produced an important increase of urinary excretion in normally hydrated *rats which drank tap water.* On the contrary, the same dose of renin, given *subcutaneously* did not modify diuresis (fig. 1 A; fig. 3 A). Increased diuresis was accompanied by an

increase in the water intake. It never failed to be produced irrespectively whether renin from rats, pigs or humans was injected. The response to daily injections of renin during a period of 2 to 3 weeks diminished progressively. After 40 daily injections a complete abolition of the diuretic action was observed. The hypertensinogen of these non sensitive animals, did not form hypertensin when incubated with renin. But when

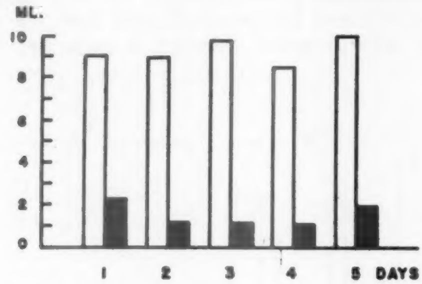


FIG. 4. — *Urinary volume expressed in ml. per 100 g. of body weight and 12 hours. Each column is the average of 6 rats which drank 1 % NaCl. White columns: Urinary excretion of rats injected daily with 0,25 ml. of renin given intraperitoneally. Black columns: Urinary excretion of rats which received an intraperitoneal injection of 0.9 % NaCl.*

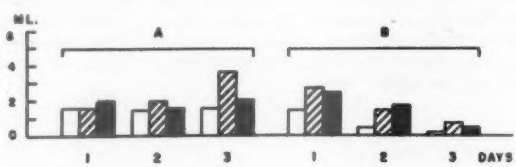


FIG. 5. — *Urinary volume expressed in ml. per 100 g. of body weight. Each column represents the average excretion of 6 rats which drank tap water. A White columns: Rats injected with 6 U of hypertensin given intraperitoneally. Stripped columns: Rats injected subcutaneously with the same dose of hypertensin. Black columns: Control rats injected with 0.9 % NaCl. B. Rats under the same conditions, injected with 1.5 U of hypertensin.*

hypertensinogen of the same animals was digested with pepsin it produced as usual pepsitensin.

Diuresis reached a maximum between the second and fourth hour after the intraperitoneal injection of renin (fig. 2). Solutions of "inactive" renin never showed any diuretic action no matter what route of administration was employed (Fig. 3 B). The diuretic effect of renin was markedly increased when the animals drank a solution of 1 % NaCl instead of water (fig. 4). In these animals when renin was given subcutaneously it induced considerable diuresis. As already insisted upon animals which drank tap water lost rather fast their ability to respond to renin with polyuria; on the contrary, in the animals that drank a solu-

tion of 1 % NaCl instead of water polyuria was maintained for a longer period. Also, in this latter group, increased urinary excretion continued even when renin was no more injected. Maximal urinary excretion was reached in animals with free water or NaCl intake during the second and fourth hour after the administration of renin. The injection of 9 units of hypertensin to animals drinking either tap water or 1 % NaCl did not modify urinary excretion; a slight effect was obtained only when 9 units were injected subcutaneously (fig. 5).

Adrenalectomy considerably diminished or completely suppressed the

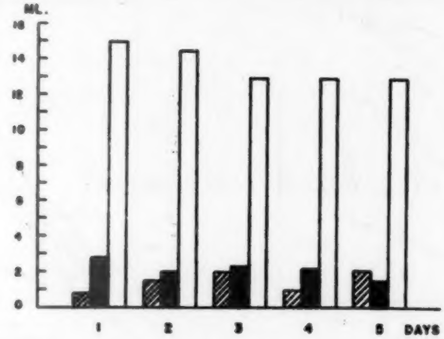


FIG. 6. — *Urinary volume expressed in ml. per 100 g. of body weight and 12 hours. All these animals drank 1 % NaCl. White columns: Normal animals injected with equal doses of renin. Black columns: Adrenalectomized animals injected with 0.9 % NaCl. All animals were injected intraperitoneally.*

diuretic action of renin given either subcutaneously or intraperitoneally to rats which drank 1 % NaCl (fig. 6).

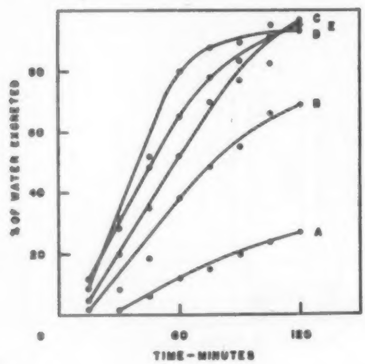
In experiments with normal *hyperhydrated rats* to wich active renin was given intraperitoneally, urinary excretion was modified in two different ways. When rats were hyperhydrated either simultaneously with the injection of renin, or one hour after, urinary excretion was considerably retarded. This effect was still evident during two hours. On the contrary in rats hyperhydrated 2 to 4 hours after injection of renin, a considerable and progressive acceleration of urinary excretion was produced (fig. 7).

Injection of "inactive" renin did not influence diuresis. The average urinary excretion did not differ from that of control groups which were injected with 0.9 % NaCl (figs. 8 and 9).

Subcutaneous injections of renin, either "inactive" or active, into hyperhydrated animals, given simultaneously with hyperhydratation or 1 to 4 hours before did not influence diuresis (figs. 8 and 9).

Adrenalectomized rats which were hyperhydrated 2 to 4 hours after the intraperitoneal injection of renin showed a considerable delay in urinary excretion when compared both to normal animals placed under the same conditions, and to adrenalectomized rats which instead of renin received an injection of 0.9 % NaCl (fig. 10). Nevertheless in some adrenalectomized animals which were maintained with 1 % NaCl during a period

as long as 2 weeks, and then were hyperhydrated 2 hours after the administration of renin, diuresis was slightly increased though it never became equal to that of normal rats. When rats adrenalectomized 20 to 30 days before were deprived of 1 % NaCl and 2 to 4 days later renin was given followed in 2 hours by hyperhydration, the antidiuretic effect was even more intense than in normal animals that received renin simultaneously with water.



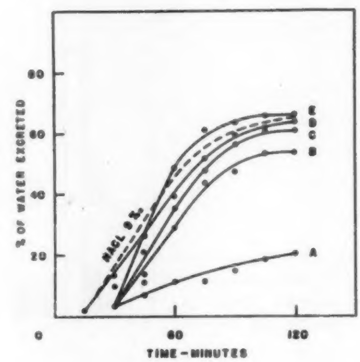


FIG. 7.—*Percentage of urine volume excreted by 4 normal rats as related to the amount of water administered by stomach tube (5 % of body weight, Burn test). All animals were injected intraperitoneally with 0.5 ml. of renin per 100 gm. A. Renin injected simultaneously with hydration. B. Renin injected 1 hour before hydration. C. Renin injected 2 hours before hydration. D. Renin injected 3 hours before hydration. E. Renin injectd 4 hours before hydration. Ordinates: % of water excreted. Abcissae: Time in minutes.*

FIG. 8.—*Percentage of urinary excretion of 6 groups of 4 rats each after using the test of Burn. A. Animals injected with 0.5 ml. of active renin per 100 gm. of body weight given intraperitoneally. B. Animals injected with equal doses of "inactive" renin, given intraperitoneally. C. Animals injected with equal doses of "inactive" renin given subcutaneously. D. Animals injected with the same dose of renin (active) subcutaneously. E. Animals injected with 6 U of hypertensin given intraperitoneally. Broken lines: these animals were injected with 0.9 % NaCl given intraperitoneally. All injections were simultaneous to hydration. Ordinates: % of water excreted. Abcissae: Time in minutes.*

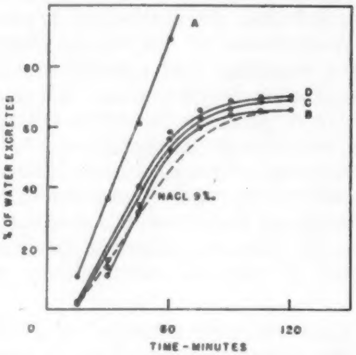
Hypertensin administered simultaneously with hyperhydratation or at the same intervals 1 to 4 hours afterwards, produced in the second hour a slight increase of urinary excretion. But this effect was similar to that produced by hypertensin previously destroyed by pepsin.

DISCUSSION

Our results give new evidence of the pronounced action renin has on water metabolism. They also show that this action depends on various

factors: the route of administration of renin, ingestion of NaCl, and the normal functioning of the adrenals.

In rats which drank tap water freely, a given intraperitoneal dose of renin produced a greater increase of diuresis than with subcutaneous administration. One may assume that the speed of absorption or destruction of renin differs according to the route of administration. But there is full evidence that NaCl increases the sensitivity of the rats to renin,



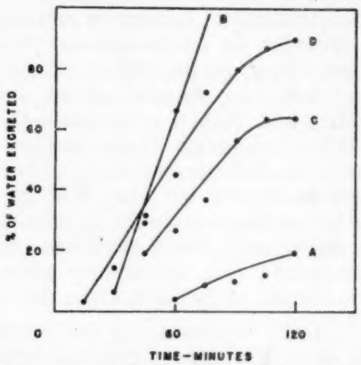


FIG. 9.—*Percentage of urinary excretion of 5 groups of 4 normal rats each, in relation with water administered using the Test of Burn. All animals were hydrated 3 hours after the injection. A. Animals injected with an intraperitoneal injection of active renin. B. Animals injected with a subcutaneous injection of active renin. C. Animals injected intraperitoneally with inactive renin. D. Animals which received a subcutaneous injection of inactive renin. Broken Lines: Control group, injected with 0.9 % NaCl intraperitoneally. In all the groups the same dose of renin was injected (0.5 ml. per 100 gm. of body weight). Ordinates: % of water excreted. Abcissae: Time in minutes.*

FIG. 10.—*Percentage of urinary excretion of both normal and adrenalectomized rats, in relation with administered water. Water was given accordingly with the Test of Burn. A. Adrenalectomized rats (4 animals) which received 0.5 ml. of renin per 100 gm. B. Normal rats (4) injected with the same dose of renin. C. Adrenalectomized rats (4) injected with NaCl (0.9 %). D. Four normal rats injected with 0.9 % NaCl. All these animals were injected with either renin or NaCl intraperitoneally 2 hours before hydration. Both normal and adrenalectomized rats received 1 % NaCl as a drinking solution from 4 days before the injections. Ordinates: percentage of water excreted. Abcissae: Time in minutes.*

in such a way that a dose of renin ineffective in animals that drink tap water produces a diuretic effect. The increased urinary excretion caused by renin in animals that receive an excess of NaCl confirms the relation existing between diuresis and sodium excretion (Pickering, 1940; Sellers, 1951). Unde the influence of renin there is a considerable decrease of tubular reabsorption of Na, Cl and water, a phenomenon that would explain the better response obtained with rats that received an excess of NaCl. A larger amount of NaCl ultrafiltered through the glomeruli would counteract reabsorption of water in the tubule.

The increased excretion of Na and Cl produced by renin shows that the latter is antagonistic to DCA and cortisone, which though stimulating diuresis and being antagonistic to vasopressin, favors reabsorption of the two ions. With regard to water excretion, renin showed to be antagonistic to vasopressin. The fact that a small effect, or no effect, was obtained in adrenalectomized rats, demonstrates that normal functioning of the adrenals is a very important factor in the diuretic mechanism of renin. This is emphasized by experiments in hyperhydrated rats. One may assume that adrenalectomy produces important alterations of the circulation, though it cannot be discarded that diminution of hypertensinogen by adrenalectomy was partly responsable of the result (Gaudino, 1944; Jarpa, 1951). It has also been reported that adrenalectomy increases the amount of an anti-diuretic factor in the blood (Birnie, 1949) and that it diminishes the sensitivity of animals to renin (Silva, 1951). Although these factors may be significant, probably what is really at stake is depletion of Na and Cl folowing adrenalectom.y This is best shown by the fact that the diuretic effect of renin in adrenalectomized animals is again increased by an excess of NaCl; on the contrary, supression of NaCl abolishes immediately the diuretic effect in adrenalectomized rats apparently already adapted to adrenal insufficiency in the course of 20 days after the operation.

Daily repetition of the injections of pig renin over a period of 30 to 40 days, produces a gradual decrease of the diuretic effect. This is probably due to the formation of anti-renin in the blood, as suggested by experiments in which hypertensinogen was titrated. Anti-renin is seemingly not formed when renin of the same species is used.

Urinary excretion in normal rats that were hyperhydrated after the intraperitoneal injection of renin shows that the diuretic effect of renin is a complex phenomenon. Two phases can be clearly distinguished: an *antidiuretic* phase which begins immediately after injection and may last up to an hour later; and a *diuretic* phase which begins in the second hour after intraperitoneal injection and may last for five hours. In adrenalectomized hyperhydrated rats only the anti-diuretic effect of renin is produced and this effect may last several hours. This phenomenon was especially marked in those animals that were deprived of NaCl. These observations suggest that the second or diuretic phase is dependent on the normal functioning of the adrenals, or on a normal amount of NaCl in the body. The diuretic effect of renin on normally hydrated and hyperhydrated rats is independent of changes in blood pressure (Croxatto, Barnafi and Villagra, 1951). This is emphasized by the fact that intraperitoneal injections of hypertensin gave no diuretic effect. But on the other hand Pickering (1940) and Brandt (1948) found with intravenous injections of hypertensin an intense diuretic effect in rabbits, and they attribute the diuretic properties of renin to the formation of hypertensin. Our results suggest that the diuretic action of renin in the rat is independent of the formation of hypertensin: the slight response of hyperhydrated animals to hypertensin was comparable to that of animals that received hypertensin previously inactivated by pepsin and devoid of pressor effect.

The anti-diuretic effect observed immediately after intraperitoneal injection of renin is probably due to formation of an excess of hypertensin which might impair circulation in the kidney by constriction of the afferent vessels of the glomeruli. One may also assume that the amount of circulating vasopressin is increased. But one may also tentatively suggest that by the renin-hypertensinogen reaction an anti-diuretic substance is liberated. This latter suggestion is based on the fact that hypertensinogen incubated with pepsin induces a strong anti-diuretic action (Croxatto, Rojas and Barnafi, 1951). But finally, one must hold in mind that renin extracts might contain impurities producing these diversified effect.

The marked effect of renin on urinary excretion together with its action on blood pressure open interesting physiological problems. It is, seemingly, the only known substance present in the body which has a clear cut diuretic action favoring the loss of water, Na and Cl. Our results seem to agree with the idea of Brandt (1948) and of Fasciolo (1951) in the sense that this enzyme is also as prominent as a diuretic factor than as hypertensive factor. In any case it seems clear that the mechanism of diuresis and renal hypertension both depend on the same enzyme.

SUMMARY

The injection of renin (12-25 U, of Braun Menendez *et al.* per 100 g. of rat) in normally hydrated animals determines a clear diuretic effect. This action is more intense when renin is injected intraperitoneally than when it is injected subcutaneously.

Diuresis induced by renin is more intense in animals which drink 1 % NaCl instead of tap water.

Renal extracts prepared in a way similar to that employed for obtaining renin, but lacking any effect on blood pressure when injected intravenously, have no diuretic effect.

Hypertensin when given intraperitoneally in doses of 1.5 U to 9 U per 100 g. body weight produced no diuretic action. When given subcutaneously 9 U induced a slight and irregular diuretic action.

Renin produced no diuretic effect in adrenalectomized rats which drank 1 % NaCl.

The effect of renin on water excretion in hyperhydrated rats is a double one. When given simultaneously or one hour before hyperhydration, it produced an anti-diuretic effect. On the contrary, when given 2 to 4 hours previous to hyperhydration, it induced a considerable increase of excretion of water.

In adrenalectomized hyperhydrated rats that were maintained with 1 % NaCl, renin produced a considerable decrease of excretion of water, even when renin was injected 2 to 4 hours before hyperhydration. Supression of NaCl increased the anti-diuretic action of renin.

Our results suggest that the action of renin on diuresis is closely associated with the amount of NaCl available in the body.

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ON CERTAIN EFFECTS OF DECAMETHONIUM (C - 10) ON THE MAMMALIAN NEUROMUSCULAR PREPARATION *

F. HUIDOBRO, L. CUBILLOS, AND C. EYZAGUIRRE **

(Departamento de Farmacología. Universidad Católica de Chile, Santiago, Chile)

A LARGE number of curarizing substances has been studied since the discovery of curare as a paralyzing agent. Special attention has been paid to *d*-tubocurarine and to decamethonium (C-10) since Barlow and Ing synthesized and tested a series of bis-quaternary ammonium compounds ([2]). Results reported in the literature indicate that both drugs produce some effects which are similar but also present many different properties ([7, 24, 25, 26 and 31]). The mechanisms by which these substances produce their effects are partly understood. Thus, it has been stressed that *d*-tubocurarine blocks neuromuscular transmission by impeding the transmitter depolarization at the end-plate. Conversely, it has been stated that synaptic blockade induced by C-10 is due to a strong and maintained synaptic depolarization ([7]).

Both C-10 and acetylcholine produce similar neuromuscular effects by depolarizing the end-plate, thus, it has been suggested that decamethonium behaves as an acetylcholine-like compound and that it should be considered a "non-hydrolyzable acetylcholine" ([25]). Therefore, most of the actions of C-10 have been explained through this similarity.

Certain phenomena dealing with "decurarization" have been studied by two of us. Comparative experiments have been performed with *d*-tubocurarine, C-10 and other paralyzing agents. From these observations it has been possible to demonstrate that C-10 presents some effects which are not similar to known properties of acetylcholine ([14]). The purpose of the present study was to gather more information in order to

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** Present address: Laboratorio de Neurofisiología, Universidad Católica de Chile, Santiago, Chile.

elucidate whether the actions of C-10 could be considered exclusively as acetylcholine-like or if they offer a wider scope of interpretation.

METHODS

Unselected adult cats were used. Anaesthesia was induced with a 25 % solution of urethane containing 3.3 % of sodium pentobarbital (1 cm^3 per kg.). Some animals were anaesthetized with 80 mg. per kg. of chloralose given intravenously. Most experiments were performed on the quadriceps femoris, the tibialis anterior being used occasionally. The femur or the tibia was fixed by means of drills as necessary. Muscular contractions were recorded on a smoked drum by fastening the corresponding tendon to the short end of a lever loaded with rubber bands. To stimulate the muscle short condenser discharges lasting 0.13 ms. were applied to the appropriate nerve through silver wire electrodes insulated with rubber. Frequency was controlled by electronic valves. Stimuli were always slightly supramaximal. The nerve was crushed proximally. Some experiments were performed on denervated muscles, prepared by aseptic section of the nerve from 8 to 12 days previously. A cannula was inserted in the trachea, if artificial respiration was necessary. Drugs were injected rapidly into the abdominal aorta below the inferior mesenteric artery, the middle sacral and the contralateral iliac arteries being ligated. Muscle action potentials were recorded from two steel needles insulated to within 1 mm. of their tips, which were inserted into the belly of the quadriceps femoris ([13]). Electroneurograms were obtained by means of silver wire electrodes placed under the peroneal nerve. The recording of end-plate potentials in normal uncurarized muscle was accomplished by using a concentric surface electrode which was gently placed on the exposed surface of the tibialis anterior. The electrode was mounted on a glass holder which could be displaced in 3 axes by calibrated micrometer screws. The skin was incised over the muscle and the edges were lifted by means of steel, pins and rubber bands in order to form a cup which was filled with mineral oil to prevent drying of the muscle. The temperature of this bath was maintained at 37° C by radiant heat from an infrared bulb. The potential changes were fed to a double beam oscillograph through five-stage capacity coupled pre-amplifiers ([12]). In all experiments frequency of stimulation ranged from 6 to 27 pulses per minute.

The drugs employed and their doses were*:

d-tubocurarine (J. Berlage & Co.) about 300 μg.
Decamethonium bromide (Burroughs Wellcome & Co.) from 5 to 200 μg. (see text).
Neostigmine (Prostigmine, Hoffmann La Roche and Prostigmina "Beta") about 250 μg.
Acetylcholine hydrochloride (Hoffmann La Roche) from 20 to 40 μg.
Atropine sulphate (Merck) 1 mg. per kg given intravenously.
Adrenaline base up to 50 μg.
Nor-adrenaline (Winthrop Stearns, Inc.) up to 50 μg.

* We wish to thank Winthrop Stearns, Inc., Burrough Wellcome & Co. and Instituto Bioquímico Beta for the drugs supplied for this investigation.

Cobefrine (Winthrop Stearns, Inc.) up to 200 µg.

Neo-Synephrine (Winthrop Stearns, Inc.) about 50 µg.

All drugs were dissolved in distilled water and injected in a volume not exceeding 0.5 cm^3. Usually the volume administered was between 0.1 and 0.2 cm^3. In some animals either several drugs were given, or one drug was given several times. In such cases some time elapsed between injections.

RESULTS

A. *Muscular twitching and fasciculation induced by C-10 and neostigmine.* — Zaimis ([31]) has pointed out that when C-10 is given in small

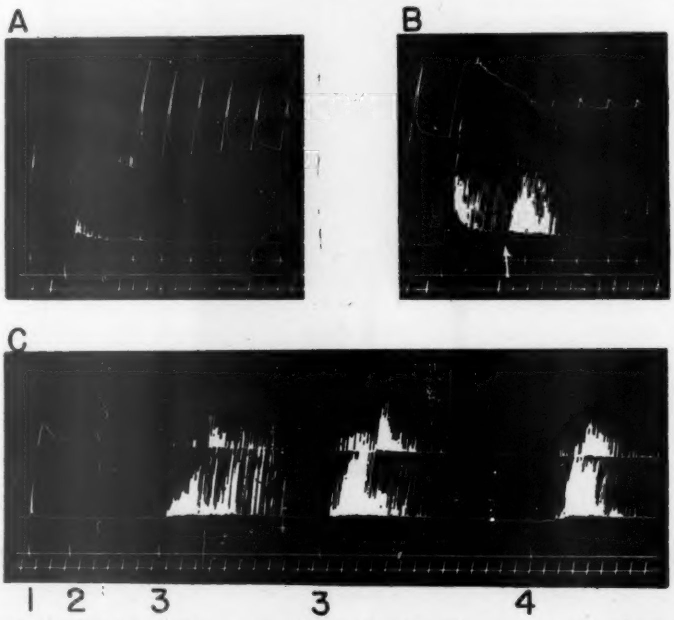

FIG. 1. — Cat weighing 3650 g., anesthetized with 80 mg. of chloralose per kg. *Upper record:* quadriceps denervated 10 days previously. *Lower record:* normal quadriceps. *Upper signal:* 10 µg. of acetylcholine given intra-arterially. *Middle signal:* C-10 injected into the abdominal aorta. *Lower signal:* Time in minutes. Variations in spacing of the time signals are due to changes in speed of kymograph. This is the case in all figures. A: *C-10 potentiation of muscular shortening induced by acetylcholine. Middle signal:* 50 µg. of C-10. B: *Paralyzing doses of C-10 depress muscular shortening induced by acetylcholine in denervated muscle, without impeding twitching and fasciculation of normal muscle. Middle signal:* 200 µg. of C-10 injected in the abdominal aorta. At arrow spontaneous respiration was paralyzed. Between A and B: 8 minutes. C: *Reappearance of twitching and fasciculation induced by C-10 after injections of Cobefrine and nor-adrenaline.* Same animal as in A and B. Between B and C: 30 minutes, and two injections of Cobefrine (50 µg. and 100 µg. respectively). During C, spontaneous respiration was completely paralyzed. (1) 50 µg. of C-10; (2) 50 µg. of Cobefrine; (3) 100 µg. of Cobefrine; (4) 25 µg. of nor-adrenaline.

doses (10 μg.) it induces an increase in the amplitude of the muscular twitch when either the tibialis anterior or the gastrocnemius is stimulated indirectly every 10 seconds. At the same time as the muscle shortens, fasciculation occurs. This phenomenon seems to be conditioned by the anesthetic used. When sodium pentobarbital was employed in our experiments, this effect was exceptional and very slight. On the contrary when the animal was anesthetized with chloralose, these effects were fre-

FIG. 2. — *Intense muscular shortening preceding neuromuscular blockade after C-10.* Cat weighing 2850 gm. anesthetized with sodium pentobarbital. Quadriceps femoris indirectly stimulated at a frequency of 17 pulses per minute. At signals: 50 μg. of C-10. Time in minutes.

quently observed in their full intensity, even after the frequency of stimulation was increased (15 to 17 pulses per minute).

Ether and barbital had an action on muscular fasciculation similar to that of sodium pentobarbital ([24]). The intensity and duration of twitching and fasciculation varied a great deal from one animal to the other. Zaimis describes something similar ([31]). In some cats this phenomenon was remarkable and it was possible to obtain intense twitching and fasciculation of long duration with doses of 200 μg. of C-10 (fig. 1 B). Occasionally when sodium pentobarbital was used, an intense muscular shortening was observed followed by disappearence of the muscular twitch. (fig. 2).

Twitching and fasciculation obtained with C-10 in doses up to 500 μg. did not last over one or two minutes (fig. 1 A). This phenomenon per-

sisted longer when it was induced with from 100 μg. to 300 μg. of neostigmine. Once twitching and fasciculation induced by Decamethonium or neostigmine vanished, a large dose of adrenaline (up to 50 μg.), nor-adrenaline (up to 500 μg.), or Cobefrine (up to 200 μg.) (figs. 1 C and 4), caused the reappearence of the phenomenon. This effect was not

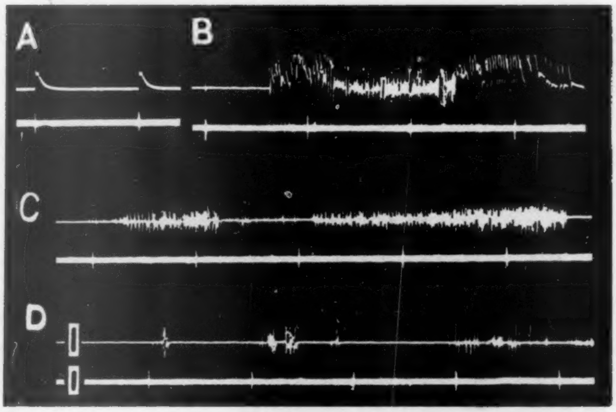

FIG. 3. — *Effect of C-10 on the electromyogram.* Upper beam: muscle action potentials. Lower beam: electroneurogram. A: control record. The peak of the muscle spike potentials is well beyond the screen because of the amplification used. B: after 50 μg. of C-10 given intra-arterially. The nerve action potentials remained unaltered while muscle spikes disappeared; 15 seconds after C-10 appears a violent outburst of repetitive discharges. C: 12 seconds after B. D: 3 seconds later. Cal: upper beam, 200 μV. Lower beam: 2 mV.

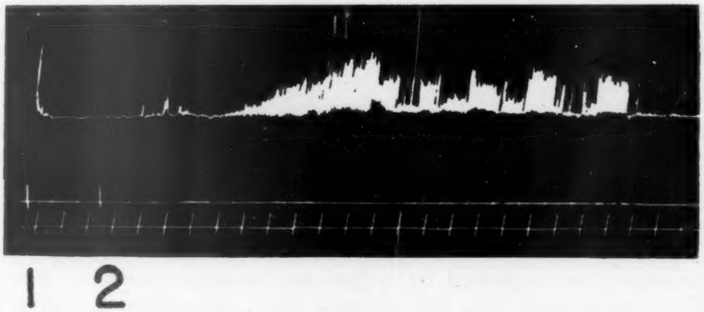

FIG. 4. — *Reappearance of twitching and fasciculation induced by neostigmine, after injections of nor-adrenaline.* Cat weighing 2200 g. anesthetized with sodium pentobarbital. Normal quadriceps femoris (1) 250 μg. of neostigmine; (2) 50 μg. of nor-adrenaline. Time in minutes.

constant but it was frequently observed. It required some time for the amines to produce asynchronous contractions. This latency ranged from one to five minutes (figs. 1 and 4). Once fasciculation reappeared, another dose of the amine exagerated the phenomenon. When the latter effect had waned the amine sometimes induced it again, if the previous injection of either C-10 or neostigmine had not been given too long a time before (figs. 1 C and 4). If a given dose of C-10 did not cause asynchronous shortening it sometimes induced it, if the amines referred to had been given previously. This effect has already been described by Paton and Zaimis for adrenaline ([25]). Adrenaline, nor-adrenaline or Co-

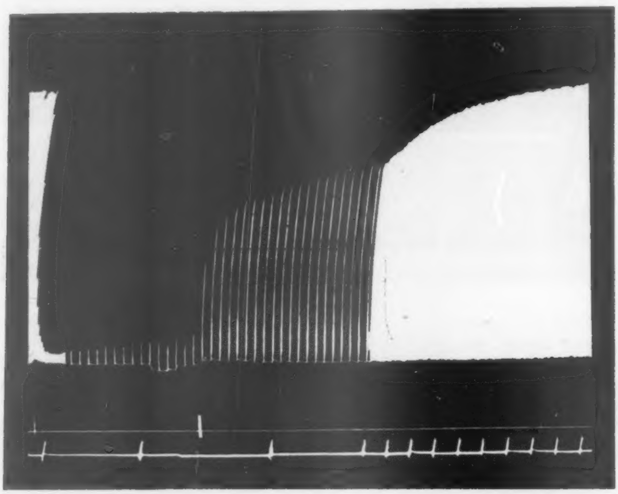

FIG. 5. — *Decurarizing action of small doses of C-10.* Cat weighing 4350 g. anesthetized with 80 mg. per kg. of chloralose. Quadriceps femoris, indirectly stimulated at frequency of 17 pulses per minute. *First signal:* 350 μg. of d-tubocurarine. *Second signal:* 40 μg. of C-10. Time in minutes.

befrine did not themselves induce fasciculation in the doses employed.

The electrical discharges induced by C-10 were recorded, special care being taken to give the drug by rapid intra-arterial injection. Results are presented in fig. 3. It was possible to observe, immediately after the injection, a violent outburst of electrical activity characterized by repetitive action potentials which waned after a few seconds. This activity was sometimes accompanied by a fast and intense muscular shortening (fig. 2). Immediately or a few seconds later, asynchronous spike potentials reappeared for a short period of time. These appeared to be the electrical concomitant of fasciculation. By careful observation of the muscle, it could be established that groups of fiber bundles were contracting at random. If a degree of synchrony was attained, then some ten-

sion developed in the form of muscular twitching. The experiment illustrated in fig. 3 represents the electromyogram of a fasciculating muscle which did not twitch. It is evident that repetitive discharges were occuring at an irregular frequency and that silent periods were present bet-

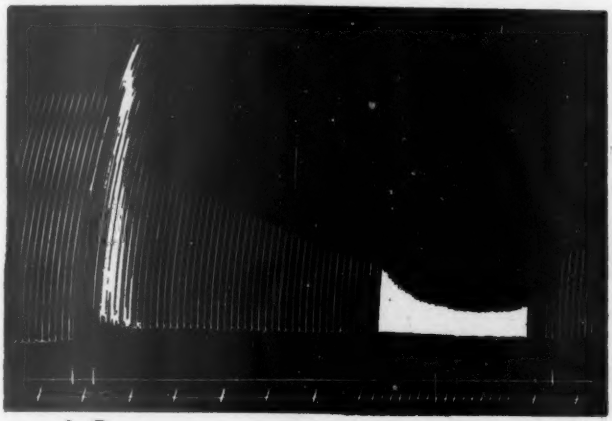

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FIG. 6. — *Decreased activity of* d-*tubocurarine after C-10. Decurarizing action of C-10.* Cat weighing 3400 g. anesthetized with 80 mg. of chloralose per kg. Quadriceps femoris stimulated indirectly at a frequency of 17 pulses per minute. (1) 20 μg. of C-10; (2) 500 μg. of *d*-tubocurarine; Last signal equal to (1). Time: 30 seconds.

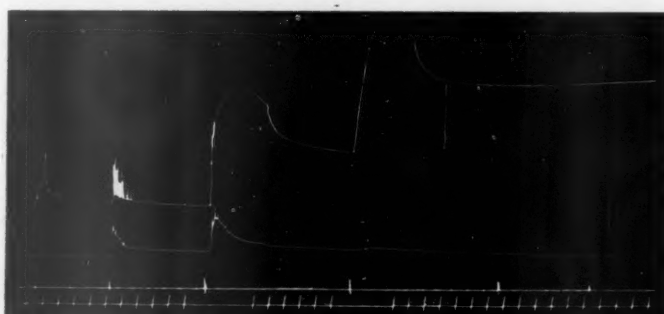

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FIG. 7. — *Increased sensitivity of denervated muscle to the action of C-10. Twitching, fasciculation and contracture.* Cat weighing 2300 g. anesthetized with chloralose, 80 mg. per kg. *Upper record:* Normal quadriceps. (1) 10 μg. of C-10; (2) 20 μg. of C-10; (3) 50 μg. of C-10; (4) 100 μg. of C-10; (5) 25 μg. of adrenaline; (6) 100 μg. of Cobefrine. Time in minutes.

ween discharges. It is interesting to notice in fig. 3 that in spite of the neuromuscular blockade induced by C-10, fasciculation of the muscle fibers persisted for some time.

B. *Anti-curare effect of small doses of decamethonium.* — Small doses of C-10 (5-40 μg.) injected into the abdominal aorta restored muscular contraction depressed by *d*-tubocurarine (figs. 5 and 6). This decurarizing acion of C-10 (see Addendum) was demonstrated both in the tibialis anterior and the quadriceps femoris, in cats anesthetized either with chloralose or sodium pentobarbital. As has been noted above, 350 μg. of *d*-tubocurarine, injected into the abdominal aorta, rapidly suppressed

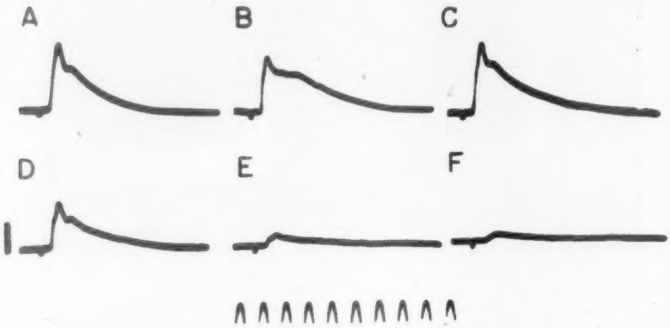

FIG. 8. — *Effect of C-10 on the end-plate potential of uncurarized muscle.* A: control record. B: after 7.5 μg. of C-10 given intra-arterially. C: 16 minutes after B. D: record taken after injecting 7.5 μg. of C-10, 30 seconds after C. E: 2 minutes after D. Thirty seconds before this record the animal received 75 μg. of C-10. F: 4 seconds after E. Cal: 5 mV. Time: 5 ms.

muscular contractions (fig. 5) if the muscle was stimulated indirectly at a frequency of from 15 to 17 pulses per minute. If before *d*-tubocurarine, small doses of C-10 were injected (20-50 μg.), larger doses of the alkaloid were necessary to produce a comparable level of curarization. In this case, the muscular contractions did not disappear rapidly, but they waned smoothly (fig. 6). When small doses of C-10 were repeatedly injected, even larger doses of *d*-tubocurarine were necessary to abolish the muscular contraction. In one instance complete curarization was obtained with 2 mg. of *d*-tubocurarine after 4.5 minutes. These findings do not agree with the results of Castillo, Phillips and de Beer ([9]).

C. *Effect of decamethonium on the muscular response induced by acetylcholine.* — The quadriceps femoris, denervated from 8 to 12 days prior to the experiment, was employed. Acetylcholine was given intra-arterially every 2 minutes. C-10 (from 7.5 to 50 μg.) was injected into the aorta from 15 to 60 seconds before acetylcholine. Under these conditions, C-10 noticeably increased muscular shortening induced by acetylcholine (fig. 1 A). Large doses of C-10 (100 to 200 μg.) capable of

producing complete neuromuscular block to indirect stimuli did not abolish, but considerably decreased the response to acetylcholine (fig. 1 B). It is interesting to notice certain relations between the muscular responses to acetylcholine and shortening caused by C-10. The muscular shortening after C-10 has been studied by Zaimis ([31]) in the denervated muscle of cat and demonstrated to be a contracture. When during contracture, low tension is being developed, an intense potentiation of the responses to acetylcholine was observed (fig. 1 A); but, when contracture developed high tension, responses to acetylcholine were depressed (fig. 1 B).

D. *Effect of d-tubocurarine and large doses of decamethonium on muscular twitching and fasciculation induced by C-10.* — Muscular twitching induced by small doses of decamethonium was not abolished by large doses of *d*-tubocurarine (fig. 6). Another remarkable finding is that large doses of decamethonium may provoke muscular twitching and fasciculation. These doses were sufficient to produce complete neuromuscular block and respiratory paralysis. Nevertheless, twitching and fasciculation persisted long after transmission was impaired. (figs. 1 and 3).

E. *Contracture induced by decamethonium on normal and denervated muscle.* — A comparative study of normal and denervated muscle was accomplished by insuring that both initial tensions and both writing levers were equal. Under these conditions, the denervated muscle was capable of shortening when responding to an intra-aortic injection of C-10 which was ineffective for normal muscle (fig. 7). Thus, this could be an instance of the "Law of denervation" ([8]). This response is a contracture according to Zaimis ([31]). Larger doses increased contracture of the denervated muscle. On the normal side, muscular shortening and fasciculation appeared (fig. 7). The mechanical response of the innervated muscle is also a contracture, judged by the following characteristics: (i) absence of electrical activity when this response was not accompanied by fasciculation; (ii) sluggish time course. It may reach half relaxation in about 2 minutes; (iii) occasionally it did not fall to the resting tension base line until 30 minutes elapsed. Even larger doses of C-10 intensified this phenomenon. Relaxation following contracture became slower and the muscle did not attain its initial tension. The normal muscle did not respond to this dose of C-10. Contracture was not modified by injections of adrenaline, Cobefrine (fig. 7) or nor-adrenaline.

F. *Effects of decamethonium on the end-plate potential of uncurarized muscle.* — The concentric electrode allowed the study of the effects of C-10 on the end-plate potential of uncurarized muscle. The tibialis anterior was indirectly stimulated at a frequency of 27 pulses per minute. Pulses used in this case were rectangular and lasted 0.5 ms. Once an area of end-plate concentration was localized by the electrode, small doses of C-10 were rapidly injected into the abdominal aorta. Thus it was possible to observe that the end-plate potential was slightly reduced in height,

but the early portion of the decaying phase became prolonged. Larger doses of decamethonium induced the well known effect on the end-plate potential by reducing its amplitude below the exciting threshold (fig. 8). It is interesting to note that a prolongation of the decaying phase of the end-plate potential was found by Eccles, Katz and Kuffler ([10]) after the administration of eserine.

Discussion

Both acetylcholine and C-10 depolarize the membrane at the neuromuscular junction. Thus, it has been pointed out that decamethonium is an acetylcholine-like substance (for references see 7). Results obtained in this paper together with observations already reported in the literature suggest that the mechanism of action of C-10 is more complex ([20, 21]).

Kuffler has demonstrated that acetylcholine acts exclusively on the end-plate, i.e., that the compound causes a local depolarization only in this area. Acetylcholine was practically ineffective when applied to end-plate free regions of the muscle fiber, even when concentrations 1000 times the end-plate exciting threshold were used ([22]). If C-10 acts only in a manner similar to acetylcholine it must be assumed that it shares these properties. But, some observations reported in the literature ([5, 20, 21]) have been interpreted as a direct action of C-10 on the muscle fiber. These findings agree with the results of the present paper. Jarcho *et al.* ([21]) have demontrated that C-10 is capable of blocking the conduction of muscle impulses obtained by direct stimulation of the denervated gracilis of the rat. If this effect is to be interpreted only as a result of local membrane depolarization, the fact that this blockade was obtained several millimeters away from the junctional zone would indicate a considerable spread of depolarization from the end-plate region. If this is the case, then C-10 depolarization could hardly be attributed to a strictly localized membrane change. Probably this observation agrees with that of Burns and Paton ([7]) who have reported that C-10 is capable of depolarizing the end-plate region of innervated muscle. Also, they indicate that this depolarization may spread a few millimeters from the junctional zones. Evidence for a non-selective action of C-10 on the innervated fiber is emphasized by the fact that decamethonium depresses the demarcation potential of normal muscle having no significative local differences with regard to end-plate zones ([21]). Furthermore, it has been demonstrated that *d*-tubocurarine, in concentrations large enough to produce complete neuromuscular block has little action on the fibrillary potentials of denervated muscle. On the contrary, if C-10 is injected, all spontaneous activity disappears completely ([20]). It has been suggested that *d*-tubocurarine blocks the avenue entrance of C-10 at the end-plate region ([21]). If this be correct, it should prevent the effect of C-10 on fibrillation because these potentials originate in the end-plate ([20]) and *d*-tubocurarine most probably impedes membrane depolarization especially at this level ([18]). Nevertheless if C-10 is given after a large dose of *d*-tubocurarine, the former drug causes complete obliteration of fibrilla-

tion. Therefore, it is highly probable that C-10 also acts beyond the myoneural junction, i.e., directly on the muscle fiber.

The following results of the present experiments are suggestive: C-10 may break a partial neuromuscular block induced by *d*-tubocurarine, when it is given in small doses (fig. 5). This fact is most remarkable because other effects of C-10 are greatly reduced, when it is given after *d*-tubocurarine [9, 24]. Since decamethonium does not affect nerve action potentials, it is possible that this action may be referred to: (i) a synaptic effect, or (ii) to a direct action on the muscle fiber. This action is probably localized beyond the endplate because Hutter and Pascoe [19] have demonstrated that small doses of C-10 do not modify the end-plate potential of curarized muscle. Therefore, the action of small doses of C-10 when acting against *d*-tubocurarine are most probably localized beyond the neuromuscular junction, i.e., on the muscle fiber. This probable mechanism of decurarization is not suprising since, as it has been stated by Hunt and Kuffler, any agent which increases muscular excitability could have an anti-curare action and that "anti-curare effects need not necessarily involve the Ach mechanism" [18].

When C-10 was given in small doses it produced muscular twitching and fasciculation of innervated muscles even after a large dose of the drug had caused complete neuromuscular block and respiratory paralysis (figs. 1 and 3). This observation suggests two possible explanations: (i) a direct effect of decamethonium on the muscle fiber, for, if neuromuscular transmission was impaired by intense and maintained synaptic depolarization, the end-plate would be inexcitable; (ii) depolarization always spreads electronically a few milimeters, thus "invading" surrounding tissue. In this case, the peripheral portion of the depolarized zone may well not be continously depolarized but alternatively repolarizing and giving rise to propagated impulses. If this be the case an explanation considering exclusively a synaptic action would be satisfactory.

The irregularity in the appearence of muscular twitching and fasciculation makes it difficult to study the effects of different drugs on this phenomenon. Nevertheless, in several experiments it has been observed that an arterial injection of large doses of *d*-tubocurarine most probably did not block muscular twitching after C-10. The mechanism of this action is difficult to understand from the present experiments, but, it is tempting to suggest that this effect of C-10 is localized on the muscle fiber unless decamethonium is blocking the action of *d*-tubocurarine at the neuromuscular junction. This is emphasized because of the fact that when this situation exists (fig. 6) even very large doses of *d*-tubocurarine fail to produce neuromuscular block. Conversely, it would be important to know whether *d*-tubocurarine would prevent the appearance of muscular fasciculation induced by C-10. This has not been attempted, because of the irregularity with which fasciculation appears.

The antagonistic effect of C-10 on true cholinesterase has been minimized in the study of the mechanism of action of decamethonium on the mammalian neuromuscular preparation, because Zaimis has indicated that this effect is negligible in the frog [31]. But how this weak anticholinesterase action may contribute to some of the effects of C-10 is still

an open question. We attempted to compare some of the actions of decamethonium with another anti-cholinesterase agent, neostigmine, which posseses, besides and action on cholinesterase, a direct action on the muscle fiber [27]. Observations reported here and those reported in the literature have been summarized in Table I. Also, eserine which is pharmacologically similar to neostigmine, induces a prolongation of the decaying phase of the end-plate potential [10]. C-10 showed a similar action (fig. 9). Nevertheless, C-10 and eserine differ in their effects on the

TABLE I

Some similar effects of decamethonium and neostigmine

	C-10	Neostigmine
1. — Twitch potentiation of indirectly stimulated muscle	+* (31)	+ (1.4,23)
2. — Potentiation of muscular shortening induced by acetylcholine	+	+ (30)
3. — Muscular shortening, twitching and fasciculation	+* (31)	+ (29)
4. — Stronger fasciculation in normal muscle	+	+ (28)
5. — Decurarizing action	+	+ (29)
6. — Potentiating effect on the activity of aromatic amines on the neuromuscular preparation	+ (16)	+ (6.16)
7. — Fasciculation is not abolished by curare	+	+ (29)
8. — Muscular repetition	+ (24)	+ (3)
9. — Anti-cholinesterase action	+ (24)	+ (28)

Twitching and fasciculation induced by C-10 and neostigmine reappeared or were increased by the action of the aromatic amines. Also, defecation, micturition and miosis which are characteristic effects of neostigmine [25], were frequently observed.

+ indicate the occurrence of the effect; number in parenthesis: the bibliographic reference.

* observation confirmed in this paper.

end-plate potential. Eserine, besides prolonging the local response, considerably enlarges it. C-10, on the contrary, decreases the height of the end-plate potential though prolonging its time course. This dual phenomenon could be analyzed on the light of our present knowledge; the delayed decay of the end-plate potential could be attributed to a protection of the transmitter [18]. The size of the end-plate potential depends on the values of resting membrane potential [15], therefore the increased height and prolongation of the rising phase observed after eserine, indicates that the time of membrane depolarization by the transmitter is lengthened [11]. The fact that C-10 slightly reduces the local potential could be explained considering that this drug causes a maintained local depolarization; therefore, when the transmitter arrives to the synaptic membrane it finds this places partly depolarized. This possibility would produce a lower voltage drop, thus a smaller potential. Nevertheless, a possible protection of the transmitter would add to the depolarization already present and therefore the end-plate potential may become prolonged. These similarities could allow the interpretation that the effects of decamethonium could also be partially due to a protection of acetylcholine.

More data are thus necessary for a complete understanding of the mechanism of action of this compound. For the time being it seems unwise to homologize C-10 and acetylcholine for many facts do not fit with

ting evidence are due to species differences [24] which are very prominent when the neuromuscular effects of decamethonium are studied.

Summary

Cats anesthetized either with sodium pentobarbital or chloralose were used throughout the experiments. Quadriceps femoris and tibialis anterior were employed.

1) Small doses of decamethonium induced twitching and fasciculation. When the muscle was stimulated indirectly at a frequency of 17 pulses per minute a potentiation of muscular contractions was observed. Larger doses sometimes produced before "curarization" an intense muscular shortening which was accompanied by electrical activity. Twitching and fasciculation sometimes reappeared under the effect of adrenaline, nor-adrenaline or Cobefrine. The latter drug also induced reappearance of muscular fasciculation produced by neostigmine.

2) Small doses of C-10 may potentiate the effect of adrenaline, noradrenaline, Cobefrine and Neo-Synephrine on the neuromuscular preparation indirectly stimulated at a low frequency.

3) Small doses of C-10 have a decurarizing action on the neuromuscular block produced by *d*-tubocurarine.

4) C-10 in doses up to 50 μg. potentiates muscular shortening induced by acetylcholine in denervated muscle. Paralyzing doses of C-10 depress the action of acetylcholine in denervated muscle.

5) Curarizing doses of *d*-tubocurarine do not abolish twitching and fasciculation induced by C-10.

6) C-10 induced contracture of both normal and denervated muscle. Denervated muscle showed to be more sensitive than the normal one.

7) Small doses of C-10 prolong the decaying phase of the end-plate potential of uncurarized muscle.

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Addendum. — After this paper was completed we learned that Hutter and Pascoe [19] demonstrated an anti-curare effect of small concentrations of decamethonium.

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THE INFLUENCE OF SODIUM IONS ON THE LOCAL RESPONSES OF AXONS

J. GARCÍA RAMOS AND E. CALVA C.

(Department of Physiology of the National Institute of Cardiology of Mexico)

SINCE Overton's (1902) studies, it is well known that a decrease of the concentration of sodium in the external fluid results in a loss of the excitability of axons. Lorente de Nó (1944 and 1947) confirmed these observations. He also found that the demarcation potential may preserve its normal value even though no sodium be present, and that the changes observed in the electrotonic potentials are probably due to the action of the substance employed to substitute for sodium while maintaining the proper osmotic pressure of the solutions.

Hodgkin and Katz (1949) observed that the amplitude of the spike potential of invertebrate axons depends on the ratio of the concentration of sodium inside to that outside the axons. They suggested that the changes of this ratio determines the disappearance of the action potential in fibers immersed in solutions that do not contain sodium. This suggestion has not been generally accepted (see Lorente de Nó, 1949).

In their study of the local responses, Rosenblueth and García Ramos (1951) concluded that the necessary and sufficient condition for the initiation of a spike potential is the development of a local response of sufficient amplitude. It was deemed important, therefore, to correlate the loss of excitability of axons, consequent to the lack of sodium, with the corresponding changes in the local responses to electric stimuli. The possibility that the substances employed to substitute for the sodium may exert an influence of their own was kept in mind. Physically and chemically inert substances were chosen and their possible effects were controlled.

We shall use the term local responses to designate the subthreshold negative variations of the membrane potential that are observed at the

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cathode of rectangular pulses when the current begins to flow, and at the anode when it ceases flowing.

METHOD

The nerves studied were spinal roots, removed from cats anesthetized with Dial (Ciba, 0.65 cm^3 per kg. intraperitoneally). The recording procedure was that described by Rosenblueth and García Ramos, 1951. Rectangular pulses were applied from one of the crushed end of the roots to an intact region. The membrane potential changes were recor-

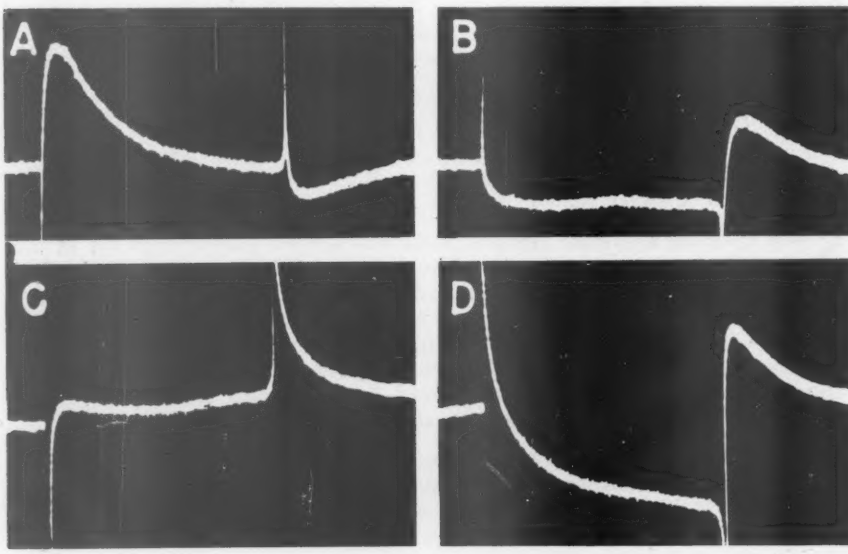

FIG. 1. — *Changes of the local responses to rectangular pulses when a nerve is immersed in a hypotonic solution (Ringer with half the normal concentration of NaCl). Duration of the pulses: 20 ms.; the sharp artifacts indicate the on and the off of these pulses. Records after balancing the passive electrotonic components (see method).*

A and B, controls, cathodal and anodal pulses, respectively, with just threshold intensity when cathodal.

C and D, after the nerve was immersed for 25 m. in the hypotonic solution. The intensity of the pulses was 5.5 times greater than in the controls.

ded from other crushed end to the point intermediate between the two stimulating electrodes where the passive electrotonic potentials have a minimum amplitude with regard to the distant recording point.

The spinal roots were tied at their ends to two glass rods. They lay on the unpolarizable electrodes (chlorided silver-Ringer-agar Ringer). The glass rods and the electrodes were fixed to a single supporting metal rod so that without changing any contacts the nerves could be inmersed into the different solutions, or placed in mineral oil for stimulation and recording purposes.

When removed from the animals, the roots were placed in a Petri dish in a standard phosphate buffered Ringer solution with the following composition per liter: NaCl, 7.96 g.; KCl, 0.42 g.; $CaCl_2$, 0.24 g.; NaH_2PO_4, 0.092 g.; and Na_2HPO_4, 0.376 g. The dorsal root was separated from the ventral one and each was used singly.

The standard solution mentioned was employed to study the reversibility of the changes in the properties of the axons treated with other

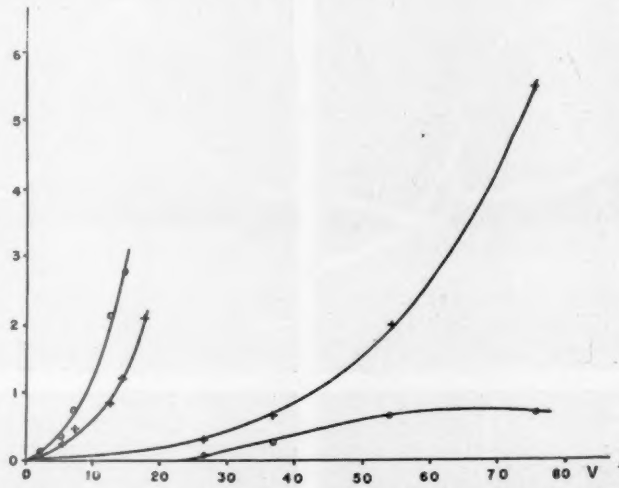

FIG. 2. — *Amplitude of the local responses (ordinates) as a function of the intensity of the pulses (abscissae). Circles: cathodal local responses; and crosses: anodal local responses. The two left hand curves show the control observations, and the two right hand curves the values obtained after the nerve had been immersed in a hypotonic solution for 25 m.*

The anodal local response became larger than the cathodal for any intensity. A propagated response was only seen when an anodal pulse was applied with an intensity five times greater than in a control observation. With the cathodal pulses no propagated responses were obtained; further intensification of the pulses led to a decrease of the local responses.

solutions. The substances used to substitute for the sodium chloride were glucose, saccharose, or lithium chloride. Only the NaCl was substituted leaving the other electrolytes with their normal concentration. Since the results were not the same in each case they will be described separately.

RESULTS

A. — *The substitution of sodium by glucose.* — The sodium chloride of the standard Ringer was replaced by glucose according to the theoretical assumption that a solution 0.28 M of this substance is isotonic with Ringer. If the nerves are used as osmometers, however, this concentra-

tion is hypotonic, since there is a swelling of the nerves. The spinal roots immersed in this solution for 30 minutes gained 20 per cent weight. In addition, the effects produced by these solutions are quite similar to those which ensue when the nerves are placed in hypotonic Ringer solution (see Lorente de Nó, 1947). These effects are as follows.

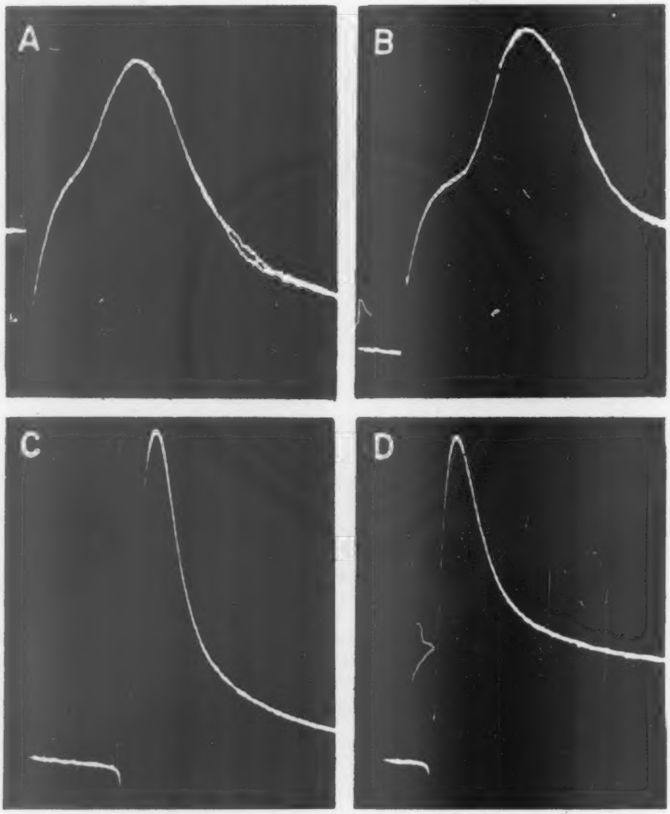

FIG. 3.—*Development of local spike potentials. A and B, controls recorded from the balance point and from a point distant 2 mm. from the pole in the extrapolar region, respectively. Cathodal pulses (10 ms.) with intensity slightly above threshold. The initial sharp excursion indicates the beginning of the pulses.*

The nerve was then immersed for 2 h. 25 m. in a solution of LiCl 0.136 M with K, Ca and the buffer phosphate system in the proportions of the standard Ringer.

C and D, spike potentials evoked at the end of strong anodal pulses (10 ms.). Records as in A and B, respectively. The speed of the sweep was the same in all cases. The spikes were much briefer and did not propagate but merely diffused electrotonically.

The threshold rises progressively. Both the local responses and the positive swings decrease (figure 1) and their time course is prolonged. One of the first changes seen in the local responses is the following. In normal nerves the responses to cathodal pulses are usually more ample

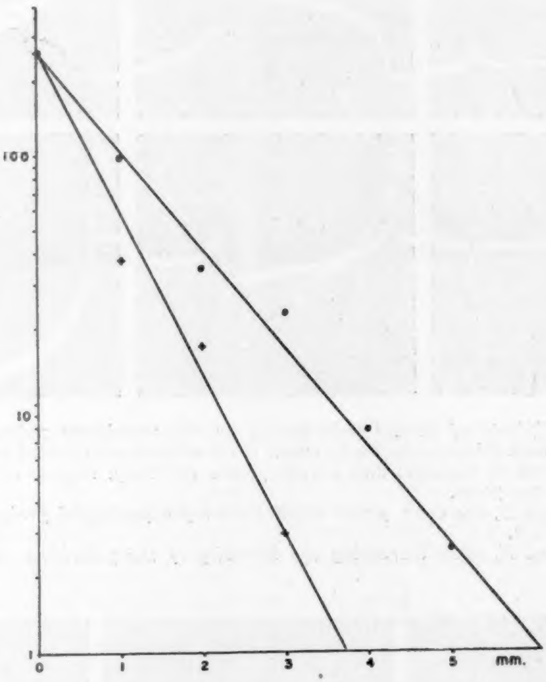

FIG. 4. — *Changes in the electrotonic diffusion of subthreshold rectangular cathodal pulses after immersion of a nerve for 50 m. in a solution of saccharose 0.278 M. Abscissae: distance in mm. from the pole, in the extrapolar region. Ordinates (logarithmic scale): amplitude of the pulses, in arbitrary units. Circles: values measured after the immersion of the nerve in the saccharose solution. Same polarizing current as before.*

than those elicited by the corresponding anodal pulses. As shown in figure 2, in the treated nerves this relationship is reversed; the anodal responses become larger that the cathodal.

The responses to subthreshold stimuli exhibit a slow component. This component is first seen at the beginning of the anodal and at the end of the cathodal pulses (fig. 1 C and D). Later it also becomes manifest at the beginning of the cathodal and at the end of the anodal pulses; the

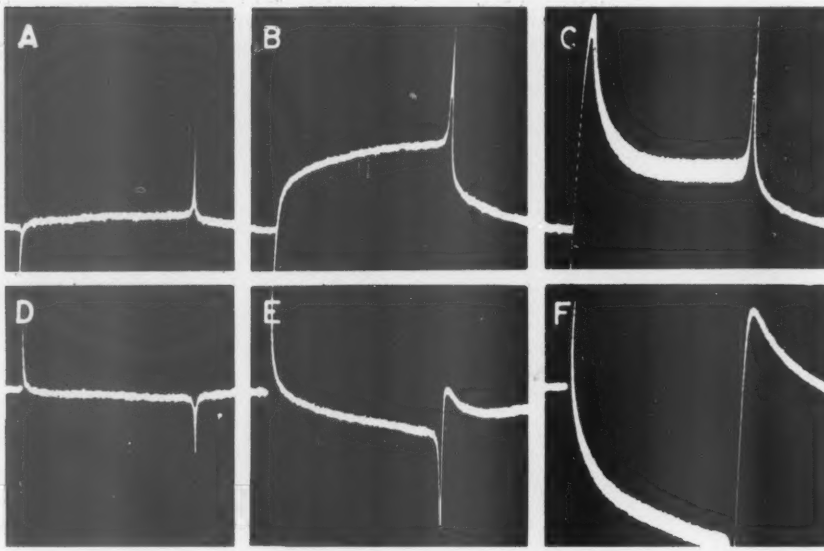

FIG. 5. — *Effects of anodal polarization on the membrane potentials of a nerve treated with Ringer solution in which the NaCl was substituted by LiCl (0.136 M).*
A and D, cathodal and anodal pulses (20 ms.), respectively. Records from the balance point.
B and E, the same pulses applied during a prolonged period of weak anodal polarization.
C and F, after increasing the intensity of the polarizing current.

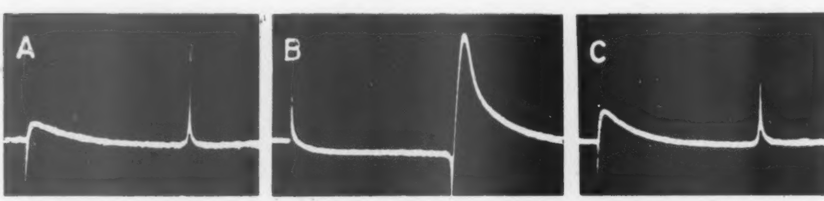

FIG. 6. — *Local responses of a nerve immersed in a LiCl solution for 2 h. 25 m. A and B, cathodal and anodal pulses with an intensity just threshold when anodal. C, threshold cathodal pulse.*

local responses are then superimposed on this slow component and their measurement becomes uncertain.

The electrotonic potentials recorded with the electrode on intact tissue near the position of optimum balance become asymmetric: the records obtained with anodal pulses are more ample than those obtained from the corresponding cathodal pulses.

The conduction velocity of the spikes decreases gradually until they are no longer able to propagate. As long as the nerves exhibit local responses, spike potentials may develop when these responses reach a sufficient amplitude. As a rule in these conditions the spike potential develops only locally; it diffuses electrotonically to neighboring regions but does not propagate. As shown in figure 3, these local spike potentials are briefer than those obtained in normal nerves.

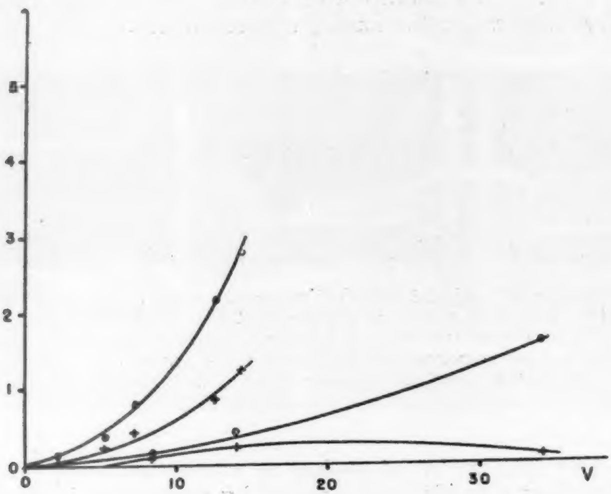

FIG. 7. — *As in figure 2, but after immersion of the nerve for 13 h. 15 m. in Ringer solution in which the NaCl was replaced by glucose (0.4 M). Note that the cathodal local responses (circles) remained more ample than the anodal (crosses) for any intensity of the stimuli.*

Anodal polarization with weak currents results in an increase of the local responses of the nerves treated with the solutions in question. When a nerve has reached a stage at which only local spike potentials develop, the propagation of these spikes to a neighboring region may be achieved if this region is polarized anodally.

The demarcation potential decreases gradually. The electrotonic diffusion along the nerves of the pulses applied decreases (see Lorente de Nó 1949). The electrotonic spread of the local responses and of the local spike potentials also decreases (figure 4).

In time the nerves become totally inexcitable. If they are not kept too long in the solutions in question the effects are fully reversible; after a relatively brief period of immersion in the standard Ringer they recover their normal properties.

The addition of glucose to standard Ringer does not lead to any of the changes mentioned above, even though nerves be placed in the Ringer-plus-glucose solution for 12 hours, and even though the solution in a small amount is kept covered by a layer of mineral oil, which produces relative anoxia.

B. — *The substitution of sodium by lithium.* — When this substitution was carried out the nerves did not swell. Their changes in properties were quite similar, however, to those reported in the previous section (see figs. 5 and 6). The observation of Gallego and Lorente de Nó (1947) that lithium depolarizes the axons, was confirmed.

FIG. 8. — *Effects of cathodal polarization on the local responses to anodal pulses of a nerve placed in a glucose solution (0.4 M) for 13 h. 15 m. A, the anodal pulse (20 ms.) does not elicit a local response. B and C, the same pulse is followed by local responses when applied in the course of a prolonged period of anodal polarization. In C, the polarizing current was more intense than in B.*

C. — *The substitution of sodium by saccharose.* — In these observations the sodium chloride was replaced by saccharose in the concentration 0.28 M. The nerves did not swell.

The changes in the properties of the axons were in general similar to those reported in section A. The following differences were noted however. The local responses to cathodal pulses did not become smaller than those to anodal pulses, on the contrary, the ratio cathodal/anodal response increased. And whereas in the observations described in section *A* anodal polarization favored the development of local responses and of spike potentials, in the nerves considered here, anodal polarization caused a further depression and cathodal polarization favored the devolopment of the responses.

D. — *Sodium-free solutions with greater concentrations of glucose.* — When instead of replacing the sodium by 0.28 M glucose a higher concentration was used (0.4 - 0.6 M) the results were as described in section *C* (figs. 7 and 8). With these concentrations of glucose the nerves did not significantly gain or lose water.

E. — *The substitution of saccharose or glucose by lithium.* — A total recovery of their normal properties was found when nerves, that had become entirely inexcitable because of their immersion in the solutions

of saccharose (0.28 M) or glucose (0.4-0.6 M), were transferred into solution in which the sodium chloride had been substituted by lithium chloride (fig. 9).

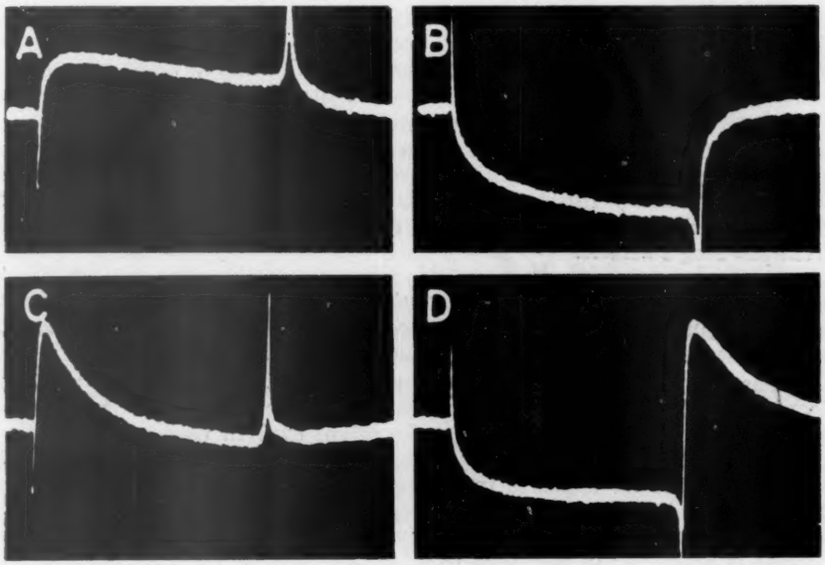

FIG. 9. — *Recovery of the prcperties of the axon membranes of a nerve placed in a Na-poor medium when transferred to a Li solution. The nerve was immersed 5 h. 30 m. in Ringer solution in which 25 per cent of the NaCl was replaced by a osmotically equivalent amount of saccharose.*
A and B, show the responses to cathodal and anodal pulses (20 ms.) respectively. The nerve was then immersed in a Na-free Li solution for 1 h. C and D, show the responses to the same pulses applied in A and B.

DISCUSSION

1) *The effects of glucose.* — Lorente de Nó (1947) noted that the changes of the properties of the axons consequent to the total or partial substitution of the sodium chloride in Ringer by a theoretically isosmotic concentration of glucose are similar to those that result from immersion in hypotonic Ringer. In his observations on the frog's sciatic nerve he did not see any swelling in the glucose solutions. Because of this observation, and in view of other considerations, he concluded that the action of glucose consists in a destruction of the axon membranes produced by some metabolite of glucose.

The present observations are not in accord with this suggestion. The cat spinal roots do swell in the glucose solutions with a concentration of 0.28 M. It is possible that the presence of the connective tissue sheath of

the frog's sciatic minimizes the swelling of the fibers. If the effects of glucose were due to a metabolite, as suggested by Lorente de Nó, these effects should be noticeable even more prominently when more concentrated solutions of this carbohydrate are employed. Our observations show that with these solutions the effects seen are no longer like those elicited by hypotonic solutions, but are exclusively due to the decrease of the sodium ion in the external medium. Furthermore, when glucose is added to standard Ringer the properties of the axons do not change even though the nerve be subjected to relative anoxia.

Our conclusion is that the axon membrane is more permeable to glucose than to sodium ions, and consequently that a solution of glucose that is theoretically isosmotic is actually hypotonic. We do not consider likely that the spinal roots of cats differ importantly in this respect from the frog's sciatic.

2) *The effects of hypotonic solutions.* — Two different mechanisms come into play in these effects: a depolarization of the axon membrane, and the decrease of sodium ions in the external fluid.

The decrease of the demarcation potential might explain the relative decrease of the cathodal local responses when compared with the corresponding anodal local responses. It also might explain why cathodal polarization depresses or blocks the propagation of impulses, whereas anodal polarization has an opposite influence. The depolarization of the membrane should be attributed to the hypotonicity of the solution, since it is not seen in more concentrated glucose solutions, in Ringer to which glucose is added, or in solutions in which the sodium chloride is substituted by saccharose.

It is possible that the decrease of the local responses may also be due, at least partly, to the depolarization, since other depolarizing agents lead to a similar loss of excitability (Lorente de Nó, 1947 an 1949).

3) *The effects of sodium-free isosmotic solutions.* — We consider here the influence of the saccharose solutions and of the solutions of glucose in concentration 0.4-0.6 M. Our observations agree with those of Lorente de Nó (1947) and those of Hodgkin and Katz (1949) in showing that the demarcation potential increases. This increase might explain the relative increase of the cathodal local responses with regard to the corresponding anodal responses (fig. 7); it also might explain the recovery of the membrane consequent to cathodal polarization.

The decrease of the spatial electrotonic diffusion seen with these solutions may be explained by the diminished conductivity of the external fluid, since a similar change is seen in nerves treated with hypotonic solutions (see Lorente de Nó, 1947). In accord with this interpretation is the fact that the phenomenon is not observed in nerves treated with solutions in which the sodium chloride is substituted by lithium chloride.

The loss of excitability of the axons is usually attributed directly to the decrease of the sodium ions of the external fluid (Overton, 1902; Lorente de Nó, 1947; Hodgkin and Katz, 1949). The present observations do not support this view. Thus, nerves that have become inexcitable

after immersion in sodium-free solutions recover their excitability when placed in lithium solutions. Again, Lorente de Nó (1949) showed that some quaternary ammonium compounds can reestablish the excitability of axons in the absence of sodium ions.

An alternative explanation is that the loss of excitability of the axons immersed in the solutions in question is due to effects on the mechanism which maintains the demarcation potential. In all the instances studied here a recovery of the fibers was obtained when the demarcation potential was shifted to its normal level. Thus anodal polarization favored excitability and propagation in the nerves that had been partially depolarized (hypotonic solutions or lithium solutions), and cathodal polarization restored the fibers in which the demarcation potential was high (saccharose or glucose isotonic solutions). Again the depolarizing agent lithium led to the restoration of the properties of the nerves which had an increased demarcation potential. Also, in the observations of Lorente de Nó (1949), quaternary ammonium ions, which augment the demarcation potential, led to the functional recovery of nerves placed in depolarizing solutions.

Thus, in the present observations the local responses developed more regularly when the demarcation potential had its normal value; any deviation from this value resulted in their decrease. The same considerations apply to the initiation and propagation of spike potentials. A generalization of this inference, however, is not justified. There are conditions in which the local responses increase although the membrane is partially depolarized, e.g., during cathodal polarization (Rosenblueth and García Ramos, 1951).

4) *The local responses and the spike potentials.* — Hodgkin and Katz (1949), found that the decrease of sodium concentration in the external fluid was followed by a decrease of the amplitude of the spike potential. Our observations however were carried out on multifibered trunks and do not allow, therefore, any definite conclusions on the changes in the magnitude of the spike potential per fiber. It appears likely that the lack of sodium is directly responsible for the fact that the initiation of spike potentials requires the development of more ample local responses than are necessary in normal nerves (fig. 2).

The decrease of the propagation velocity of the impulses may be due to two factors. The first is the fact mentioned above that the initiation of spike potentials requires an ample local response in the absence of sodium ions. As shown by Bullock and Turner (1950), when a nerve impulse propagates, the development of the spike at any given region is probably preceded by the development of a local response. The firing of the spike will occur sooner when a small local response is sufficient than when a large one is necessary. The second factor that may slow conduction velocity is the reduction of the electrotonic diffusion. In these conditions the spike potentials will be briefer in time and occupy a smaller stretch of nerve. These changes will result in a decrease of the local response at the segment ahead of the propagating spike.

An additional fact that supports the inference that local responses are involved in the propagation of spikes is the increase of the amplitude and conduction velocity of the impulses in segments polarized anodally or cathodally when this polarization restores the normal value of the demarcation potential and increases the amplitude of the local responses of these segments.

Summary

The local responses to rectangular electric pulses were studied in cat spinal roots treated by immersion into various solutions poor in sodium ions.

When the NaCl of Ringer solution is replaced by glucose 0.28 M the nerves show changes similar to those seen when treated by hypotonic solutions. The local responses decrease (fig. 1), particularly those to cathodal pulses (fig. 2). Local spike potentials may develop (fig. 3). The spatial spread of electrotonic diffusion decreases (fig. 4). The substitution of NaCl by LiCl leads to similar effects (figs. 5 and 6).

Some of the changes noted above do not appear if the NaCl is replaced by saccharose (0.28 M) or by greater concentrations of glucose (0.4-0.6 M; figs. 7 and 8). Nerves that have become inexcitable in these solutions may recover if placed in LiCl Ringer (fig. 9).

It is inferred that the 0.28 M glucose solutions are hypotonic, and that the changes they elicit are due both to the depolarization of the axon membrane and to the decrease of Na ions in the external fluid. Isotonic Na-poor solutions lead to an increase of the demarcation potential. The loss of excitability is attributed directly to the changes of the demarcation potential, not to the decrease of the Na concentration.

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